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MYCOLOGIA

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XLVI JULY–AUGUST, 1954 No. 4

INTERSPECIFIC HYBRIDS AND THE CYTOGENETICS AND CYTOTAXONOMY OF EUALLOMYCES

RALPH EMERSON [1] AND CHARLES M. WILSON [2]

(WITH 15 FIGURES)

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[1] University of California, Berkeley, California. Much of the genetic work in this paper was done between 1937 and 1939 during the tenure of a National Research Council Fellowship at the Botany School, Cambridge University, England. Later cytogenetic studies were made on a John Simon Guggenheim Memorial Fellowship at Harvard University, 1948–1949. Terminal phases of the investigation were supported by a research grant (RG-2149R) from the National Institutes of Health, Public Health Service.

[2] McGill University, Montreal, Canada. The investigations of polyploidy as well as various aspects of hybrid cytology were done on a National Research Council Fellowship at the University of California, Berkeley, 1950–1951.

We are indebted to the many kind people who have given us encouragement, help, and advice during the long course of this work. It is a particular pleasure to express our deep gratitude to Professor W. H. Weston under whom we both studied at Harvard University. We also wish to thank Dr. Arif S. EL-Ani for his critical and painstaking preparations of text figures 1, 3, 5, 11 and 13.

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In a number of ways the fungi lend themselves particularly well to genetic research. The literature of the past thirty years clearly shows the significance of the contributions that investigations of the fungi have made to our over-all understanding of the mechanisms of inheritance. And, conversely, genetics has served as a valuable tool for the mycologist, enabling him to clarify basic aspects of the life histories, reproductive processes, and general behavior of the major groups of fungi. Brilliant studies have been made with various genera of the Basidiomycetes, Ascomycetes, and terrestrial Phycomycetes. Hitherto, however, there have been only brief reports of any genetic work on the aquatic members of the Phycomycetes, organisms in which the great diversity of life cycles and sexual mechanisms offers particularly interesting possibilities. Intraspecific crosses have provided clues to the sexual behavior of *Dictyuchus monosporus* (Couch, **1926**), *Phytophthora faberi* (Ashby, **1922**; Gadd, **1924**, **1927**), *Achlya bisexualis* and *A. ambisexualis* (Raper, **1936**, **1940**), and *Sapromyces elongatus* (Weston, **1938**; Bishop, **1940**), but in no instance was a real genetic study made. Salvin (**1942**) described intergeneric matings between *Dictyuchus* and *Thraustotheca* but did not succeed in germinating the hybrid oospores and so could not analyze the progeny of the cross.

After reporting sexuality in *Allomyces*, Kniep (**1929**) visualized at once the exceptional opportunities for hybridization offered by this genus with its anisogamous, motile gametes and its striking alternation of gametophytic and sporophytic generations. In fact he had already undertaken hybridization experiments (Kniep, **1930**) when his untimely death early in 1931 ended an extraordinarily productive career and deprived us of this contribution.

In 1936 the senior writer of the present paper made the first of a

series of interspecific crosses between *Allomyces javanicus* and *A. arbuscula*. Preliminary results of this work were later briefly summarized (Emerson, **1941**, p. 98) in a monographic treatment of the genus. At that time, although many individual hybrids had been made, not all of them had been analyzed. The behavior of certain types of crosses was not understood, and the characteristics of the F_2 generation were still not clear. Cytological demonstration of the reduction divisions in *Allomyces* by the junior writer not only opened the way for a detailed study of meiosis (cf. Wilson, **1952**), but also provided the basis for a sound understanding and interpretation of the earlier genetic work. Furthermore, the recent discovery (Wilson, **1952**) of polyploidy in the subgenus *Euallomyces* has an immediate bearing on genetic investigations and can ultimately help to clarify the evolutionary relationships of the different species and varieties.

The aims of the following paper are therefore fourfold: (1) To present the genetic work which has been done on the inheritance of sexual characters. (2) To describe the correlations which have been found between the behavior and characteristics of the hybrids and the number and behavior of their chromosomes. (3) To report the occurrence of a series of polyploid strains. (4) To show what these genetic and cytological observations indicate regarding the cytotaxonomy of the species.

MATERIALS AND METHODS

Life cycle and structural features of Euallomyces—All of the genetic work reported here was done with the two species, *A. javanicus* Kniep and *A. arbuscula* Butler, which constitute the subgenus *Euallomyces*. The basic morphological, developmental, and cultural features of these forms have been described in an earlier work (Emerson, **1941**). Knowing now that meiosis occurs in the resistant sporangia (Emerson and Wilson, **1949**; Wilson, **1952**), the salient features of the normal life cycle of *Euallomyces* (see Emerson, **1941**, Fig. 4) can be summarized as follows. Haploid spores from resistant sporangia form haploid, hermaphroditic sexual plants which bear female and male gametangia. The female gametangia are nearly colorless while the male gametangia are colored bright orange by gamma-carotene (Emerson and Fox, **1940**). Motile gametes are released from the gametangia. The female gamete is colorless and about 10 μ in diameter; the male gamete contains carotenoid pigment and is about 6 μ in diameter. The motile zygotes produced by syngamy develop into diploid, sporophytic plants which bear zoosporangia and resistant sporangia. The zoosporangia are thin-walled

and nearly colorless. They release diploid zoospores, about 12 μ in diameter, which develop into sporophytic plants. The resistant sporangia are thick-walled, pitted, and colored brown by melanoid pigments (Emerson and Fox, **1940**) in their heavy middle wall. They release haploid zoospores, about 10 μ in diameter, which develop into gametophytic plants. There are then, aside from the biflagellate zygote, four kinds of uniflagellate structures: male gametes, female gametes, haploid zoospores, and diploid zoospores. The last three can be distinguished only by their origin and behavior. In all subsequent discussions the two types of spores and sporangia will be designated by the terms previously used by Emerson (**1950**, p. 189): haploid zoospores are called *meiospores* formed in a *meiosporangium* (resistant sporangium), while diploid zoospores are called *mitospores* formed in a *mitosporangium* (thin-walled zoosporangium).

There are a number of features of the above cycle and the structures involved that must be kept clearly in mind in the discussion of genetics that is to follow. (1) Meiospores, mitospores, and gametes are normally uninucleate. (2) Gametophytes are haploid, hermaphroditic, and self-fertile; therefore, when selfed, they give sporophytes in which the diploid nuclei contain a set of paternal chromosomes numerically, structurally, and genetically identical with the maternal chromosomes. In other words, whenever a gametophyte is selfed, the resulting sporophyte is completely homozygous in all characters. (3) Each filial generation is actually composed of two separate parts, the diploid sporophyte arising from the zygote, and the haploid gametophyte arising from meiospores.

We come now to a consideration of the naturally occurring differences which could be used in a genetic study of *Euallomyces*. Among the known filamentous fungi, *Allomyces* is the only one that has an extensive mycelium in which true diploid nuclei—in contrast, for instance, to the dikaryotic condition of the Basidiomycetes—multiply by mitosis. It was recognized at the start of the present study, therefore, that a genetic analysis of sporophytic characters would be particularly interesting since dominance relations like those exhibited in higher plants and animals might be investigated. The fundamental similarity, however, of all the basic structural features of the sporophyte of *Euallomyces* has been brought out (Emerson, **1941**) in a detailed comparison of 43 separate isolates of *A. javanicus* and *A. arbuscula* obtained from widely scattered regions of the world. While the inheritance of meiosporangium size (cf. Emerson, **1941**, Fig. 9 and Table 4) might be treated statistically, it would be an arduous task with results which would perhaps be of doubtful significance.

Attention was directed almost immediately, therefore, to the gametophytes of *Euallomyces* which exhibit a major, clear-cut morphological difference. It is this difference, in fact, which serves to distinguish the two species of the subgenus. The primary gametangia are usually borne on the hyphal tips in pairs, a male and a female together. As shown in FIG. 7 the male cell is terminal in *A. javanicus* var. *macrogynus*, or epigynous, while in *A. arbuscula* the male cell is subterminal, or hypogynous. After determining in 1936 that crosses could be effected between these two species and that viable offspring could be obtained, the subsequent genetic work to be presented here was centered largely upon the inheritance of this character which we may term *gametangial arrangement*, with E to designate epigyny and H to designate hypogyny.

Isolates and general cultural methods—All of the isolates of *Allomyces* used in the present study are listed in TABLE VIII. Data on the origin and characteristics of a majority of them were reported by Emerson in 1941. Details about the ten or so strains isolated thereafter (designated * in TABLE VIII) are not germane to the present discussion and will be published at a later time.

The basic cultural methods employed have also been described (Emerson, **1941**). The standard culture medium (YpSs) used for growing gametophytic and sporophytic thalli was composed of 4 g powdered yeast extract (Difco), 15 g soluble starch, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 20 g agar per liter of water. When it was being prepared for streaking zoospores or zygotes, to obtain single germlings, this medium was diluted to ½ strength and poured in standard (10 cm) petri dishes. When the agar had solidified, three or four drops of sterile water were placed in the middle of a plate; the swarmers were pipetted into this water which was then spread evenly over the agar surface with a small glass rod slightly curved at the tip.

Special methods used in the genetic work—It will now be well to go over point by point the exact procedures used in the genetic work.

1) Isolation of 4 types of gametangia: Mature gametangia are readily removed from agar cultures by scraping the surface growth gently with a flattened transfer wire. In this way a small mass of gametangia of each of the two species is placed in separate petri dishes (6 cm) of sterile water. On slight stirring with the transfer wire the masses fall apart. Most of the gametangia become dislodged from their parent hyphae and lie on the bottoms of the dishes fairly evenly distributed, singly, in pairs, or in clusters. Under a dissecting microscope it is an easy matter to distinguish the male and female cells, and the single ones of either sex can be picked up one by one in the capillary stream of a glass pipette

drawn out to a suitable bore. In 10 or 15 minutes E-type females and E-type males of *A. javanicus* and H-type females and H-type males of *A. arbuscula* can be segregated into 4 hollow ground slides each containing about 0.3 ml of water and held in a moist chamber. Usually 5 to 10 females and 50 to 100 males of each species were isolated in this fashion.

2) Making the crosses: About 60 minutes after the gametangia are initially placed in water the gametes begin to emerge and crawl or swim about. They, too, can be seen clearly under a high power dissecting microscope (60–80 magnification). With pipettes drawn out to an even finer bore (about 20 μ diam.) individual female gametes can be picked up and transferred to hanging drops. These drops were usually made with a diameter little larger than the low power field of the compound microscope so that all parts of the drop could be quickly examined by focusing. When 10 or more hanging drops had been prepared, each with one to five E-type female gametes, a goodly number of H-type male gametes was added to each drop. Similarly, H-type female gametes were isolated and E-type males were added. The reactions between the gametes of opposite sex from different species could be observed, the process of syngamy could often be seen, and the biflagellate zygotes could usually be found thereafter. While the above method was used for making nearly all crosses from 1 to 102, hanging drops were not prepared for crosses 103–112. Instead E-type male gametangia were placed directly with H-type females, and *vice versa*, in hollow ground slides, and syngamy was allowed to take place right in these slides.

3) Isolation of hybrid zygotes: In the first 28 crosses, nutrient for the zygote to make its initial development was provided by a very small bit of hemp seed included right in the hanging drop. While hybrid germlings would grow sturdily under these conditions, the piece of seed and the cells and droplets of oil which inevitably became loosened from it often interferred seriously with microscopic examination of gamete- and zygote-behavior in the early stages of the cross. Nutrient was provided, therefore, in almost all subsequent crosses up to 102, by spreading a thin layer of ½ strength YpSs agar over the central area of each cover slip before making the hanging drop. The agar film did not interfere at all with microscopic observations, and the zygotes had sufficient nutrient to form healthy plantlets which could be transferred individually to fresh medium after 48 hours or more. In crosses 103–112, obtained by mass-mixing of gametes, after allowing about 3 hours to insure syngamy, 40–50 zygotes were pipetted onto agar plates and streaked as previously described. After 24–48 hours the young hybrid plants were cut out and transferred separately to fresh medium.

4) Germination of meiosporangia: In order to obtain gametophytes from hybrid sporophytes it was necessary to get mature meiosporangia, free of any viable mitosporangia, and induce them to germinate in quantity. This was done in two different ways. In the early work the standard procedure (Emerson, **1941**, p. 88) consisted of harvesting meiosporangia from agar cultures, 4 to 8 weeks old, and drying them for periods of a few weeks or more on strips of filter paper. When such dried material was placed in water, anywhere from 10% to 95% germination of meiosporangia usually occurred within a period of 8 to 24 hours. Since all of the mitosporangia had been killed by desiccation, there were no mitospores present, and the meiospores could be used directly to obtain gametophytic plants. During the past two years, with a more fundamental understanding of the maturation of meiosporangia (Wilson, **1952**) it was possible to use a simpler procedure giving consistently higher and more uniform meiosporangium germination. Meiosporangia were allowed to reach full maturity on the slants where they had been produced. The time varied considerably for different strains but was ordinarily no longer than 6 or 8 weeks. As soon as cytological examination or germination tests revealed that a majority of the meiosporangia were mature, they were scraped from the cultures and placed in water. Not having been fully dried, viable mitosporangia usually accompanied the meiosporangia. It was a simple matter, however, to segregate the two types of reproductive structures by the method employed to segregate male and female gametangia (see above). Mass germination of meiosporangia was completed in 1½ to 3 hours.

5) Isolation of single gametophytes: In the early work, when meiosporangia were being dried and a high, uniform germination could not be relied upon, they were allowed to discharge meiospores in water cultures baited with hemp seeds. After 2 or 3 days, when the gametophyte plants were starting to form gametangia, hyphal tips were taken from various parts of the growth and plated on nutrient agar. A much more positive and satisfactory method was used in subsequent studies when mass germination of meiosporangia was assured. Meiospores were pipetted directly onto agar plates and streaked in the usual manner. After 24–48 hours single gametophyte germlings could be selected and transferred to fresh medium.

6) Measurements of viability: After the early phase of these studies it became apparent that the viability of the meiospores from F_1 hybrids was but a small fraction of that of meiospores from the parental strains. Considerable effort was made therefore to obtain at least semiquantitative determinations of the fertility of the various genetic strains being used. With the development of good methods for mass germination of meio-

sporangia, measurements of the viability of meiospores became relatively simple, and a standard procedure was worked out. To establish the general range of viability of a given strain, about three plates were streaked with meiospores, one with 15–20 spores, one with 50–100, and one with 500 or a thousand. The number of plants which developed in each plate of the series gave a good indication of the range of viability. Thereafter, to determine the percent viability, one to several additional plates were streaked with the most suitable number of spores. The spores were counted under the dissecting microscope as they flowed into a capillary pipette. The higher counts were less accurate but were sufficiently precise to establish the marked differences in viability that occurred.

7) Method of scoring epigyny and hypogyny: It also became evident that quantitative expression of the character, gametangial arrangement, would be important in analyzing the products of crosses between epigynous and hypogynous species. The method employed was essentially the same as that used to distinguish between the three varieties of *A. javanicus* (Emerson, **1941**, p. 113). Gametophyte plants were grown on agar for one to four weeks until a good crop of gametangia had been produced. A random sample of gametangia was then scraped from the surface in the usual way and mounted in water on a slide. With a little practice most of the terminal pairs of gametangia showing epigyny or hypogyny could be readily recognized by their color, morphology, and point of attachment. Counts of 50 or 100 pairs served to establish the E:H ratio. In certain instances where more precise values were required 500 to 1000 pairs were counted.

8) Designation of hybrid strains: In order to keep track of the various crosses and their subsequent progeny, some scheme of numbering had to be used. The problem is complicated by the fact that, as stated previously, each complete filial generation is composed of two separate parts, the diploid sporophyte and the haploid gametophyte. Although it is somewhat cumbersome, the following system has proved generally satisfactory. Each filial generation is designated by a number in brackets, and the sporophytic and gametophytic phases are shown by the letters S and G. Thus (1) SG is the entire first filial generation, (2) SG is the second, and so forth. To distinguish particular crosses, each S or G in each generation has an individual number, and the entire pedigree of a particular culture is shown by its key label. For example, (1) S55G58 (2) S4G3 will mean: gametophyte 3 derived from second generation sporophyte 4 derived from gametophyte 58 which was derived from the original F_1 sporophyte 55.

One further complication occasionally had to be added. Whenever a given sporophyte has been reproduced asexually by single mitospores (diploid zoospores), this will be indicated by the letter Z in square brackets following the sporophyte number. (1) S34[1Z] indicates, therefore, that F_1 sporophyte 34 was carried through one mitospore cycle, whereas (1) S35[5Z] shows that hybrid S35 was carried through five successive asexual cycles with single mitospores. Subsequent letters and numbers within the square brackets simply serve to distinguish individual mitospores, i.e., [1ZA4] means mitospore 4 from mitosporangium A of the first mitospore cycle.

THE INTERSPECIFIC CROSSES

Behavior of gametes and proportion of "takes"—Although records were frequently kept concerning the reactions of the gametes each time crosses were made, the observations are largely subjective and not susceptible to detailed analysis. Nevertheless, a few points which emerged are worth noting here. Control slides, set up with hanging drops of selfed material, i.e., E♀ × E♂ or H♀ × H♂, showed that the reaction between gametes of the same species under the conditions employed was immediate and very characteristic. Males were usually found in contact with the female almost as soon as the slide could be placed under observation, zygotes could be detected within 10 minutes or less, and thereafter the remaining males swam aimlessly about the drop. This sequence of behavior may then be termed *typical*. In most of the interspecific crosses prepared, the reaction of the gametes was much the same. A rather longer period (up to an hour) was usually required before zygotes could be found. This indicated some degree of incompatibility, but otherwise gamete behavior was essentially similar to that of the controls. TABLE I shows that in all crosses of E♀ × H♂ this fairly typical reaction occurred. In the reciprocals, H♀ × E♂, there was also essentially typical behavior when Ceylon *1* was used. When Philippine Islands *1* (P.I. *1*) and Burma *1A* were used, however, the reaction was nearly always different. Two patterns were apparent: either there was no evident reaction at all between the gametes, the males appearing to pay no attention to the female though often in chance close proximity, or there was a strong reaction, the males clustering about the female in large clumps. In the latter instance, however, the males appeared unable to effect fertilization, because the clumping continued for periods up to five or six hours. We have no definite knowledge at present of the precise reasons for the two kinds of atypical behavior. At all events,

in only three of the crosses (12, 41, and 42, TABLE II) made with these parents (P.I. *1* or Burma *1A* ♀ × Burma *3* ♂) could zygotes be detected despite search for them, at intervals, up to six hours. It was therefore likely that syngamy occurred very rarely in such combinations under the conditions employed. Further evidence on this point came from analyses of the progeny of these combinations to be given in the section on parthenogenesis below.

TABLE I

THE REACTION OF THE GAMETES AND THE PROPORTION OF "TAKES" IN INTERSPECIFIC CROSSES OF *EUALLOMYCES*

Parents	Gamete-reaction	Proportion of "takes"	
		Sporophyte germlings obtained per ♀ gametes used	
		Nos.	%
Controls Selfed			
A. javanicus (E) ♀ × *A. javanicus* (E) ♂	Typical	45/50	90
A. arbuscula (H) ♀ × *A. arbuscula* (H) ♂	Typical	27/40	68
Crosses			
A. javanicus (E) ♀ × *A. arbuscula* (H) ♂			
P.I. *1*	Typical	7/41	17
Burma *1A*	Typical	12/30	40
Ceylon *1*	Typical	9/235	4
A. arbuscula (H) ♀ × *A. javanicus* (E) ♂			
P.I. *1*	Atypical	17/52	33
Burma *1A*	Atyp. or Typ.	9/54	17
Ceylon *1*	Typical	30/67	45
Controls ♀s Only			
A. javanicus (E) ♀	—	0/200	0
A. arbuscula (H) ♀			
P.I. *1*	—	84/295	29
Burma *1A*	—	3/30	10
Ceylon *1*	—	1/ca. 300	<1

Typical indicates that the gametes of opposite sex showed a reaction to each other within a short period, zygotes were found soon after, and no further reaction was evident.

Atypical indicates that the gametes showed either no reaction to each other or a strong reaction that continued for an extended period. In either instance zygotes could not be detected.

The last column of TABLE I summarizes the records which were obtained on the number of "takes," that is to say the number of sporophytic plants obtained per number of female gametes used. Delicate structures such as gametes of *Allomyces* may well be subject to injury as a result of pipetting, streaking, and so forth, and they probably respond to subtle changes in their environment. Inasmuch as the sequence of crossing

experiments was made over a considerable period of years, in different laboratories, and in different ways, with no rigid standardization of conditions, detailed conclusions cannot be drawn from the figures given. A number of general points appear to be quite well established however. First, selfed material, E♀ × E♂ and H♀ × H♂, gave consistently high "takes" showing that the treatment which the gametes received and the conditions used were satisfactory for normal sexual fusions and subse-

TABLE II

SUMMARY OF INTERSPECIFIC CROSSES

♀ Parent	♂ Parent	Each number represents a first generation sporophyte,* i.e. (1)S
A. javanicus (E) × *A. arbuscula* (H)		
Burma *3*	P.I. *1*	1, 2, 3, (4), 5, (39), (40)
Burma *3*	Burma *1A*	15, 16, (17), 18, 31, 32, 33, 34, 35, 36, 37, 38
Burma *3*	Louisiana *1*	48
Burma *3*	Ceylon *1*	(72), 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85
Burma *1Da*	Ceylon *1*	103, (104), (105)
Java *1*	Ceylon *1*	19, 20, 21
A. arbuscula (H) × *A. javanicus* (E)		
P.I. *1*	Burma *3*	*6, 7, 8,* (9), *10, 11,* (29), (30), *86, 87, 88, 89, 90,* (91), *92, 93, 94,* (95), *96, 97, 98, 99, 100, 101, 102*
Burma *1A*	Burma *3*	12, (13), *14,* 41, 42, *43, 44. 45, 46, 47*
Ceylon *1*	Burma *3*	49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71
Ceylon *1*	Burma *1Da*	106, (107), 108, (109), 110, (111), (112)
Ceylon *1*	Java *1*	(22), 23, 24, 25, 26, 27, 28

* Italics indicate the strains that gave exclusively H-type gametophytes (see text); parentheses indicate strains which were not analyzed; all other strains gave I-type gametophytes and usually E and H as well when a sufficiently large population was analyzed.

1–72 (excepting 12 and 22–30) were single crosses made with one isolated female gamete in each hanging drop.

12, 22–30, and 73–102 were crosses made with two to ten female gametes in the same drop.

103–112 were made with many female gametes; the zygotes were then isolated by streaking after syngamy and before germination.

quent growth of the zygotes. Second, although as might be expected the interspecific crosses showed a lower percentage of "takes" than the selfed material, they are of the same general order of magnitude and range, with one exception, from 17 to 45 percent. Third, despite the typical reaction of the gametes in all crosses with Ceylon *1*, far fewer viable zygotes were obtained when this strain was the ♂ parent than when it was the ♀. A discussion of the figures on parthenogenetic devel-

TABLE III

GAMETANGIAL ARRANGEMENT IN PARENTAL AND REPRESENTATIVE F1 GAMETOPHYTES

Isolates	Pairs counted	E, %	H, %
A. arbuscula (H)			
Burma *1A*	1000	0	100
Ceylon *1*	1000	0	100
Louisiana *1*	1000	0	100
Philip. Isls. *1*	1000	0	100
A. javanicus (E)			
Burma *1Da*	1000	100	0
Burma *3*	4000	100	0
Java *1*	3000	99	1
E ♀ × *H* ♂			
(1)S31G1	33	100	0
G3	100	64	36
G2	36	0	100
(1)S31[1ZA4]G4	124	99	1
G1	133	89	11
G3	156	0	100
(1)S34G1	97	96	4
G3	98	75	25
G2	101	4	96
(1)S34[1ZB5]G2	71	100	0
G3	84	35	65
G1	122	0	100
(1)S35[5Z]G2	100	100	0
G8	100	92	8
G6	100	56	44
G14	100	22	78
G11	100	1	99
(1)S103G2	100	98	2
G4	100	88	12
G6	100	62	38
G5	100	51	49
G7	100	38	62
H ♀ × *E* ♂			
(1)S49G3	12	100	0
G6	24	96	4
G5	32	85	15
G2	33	35	65
G1	121	0	100
(1)S55G3	50	100	0
G25	50	78	22
G10	50	56	44
G24	50	31	69
G21	50	0	100
(1)S106G36	100	100	0
G38	100	69	31
G28	100	40	60
G31	100	22	78
G41	100	0	100
(1)S108G4	100	100	0
G11	100	79	21
G14	100	44	56
G5	100	12	88
G18	100	0	100
H ♀ (× *E* ♂)*			
(1)S*11*G1	100	0	100
G2	50	0	100
(1)S*43*G1	199	0	100
G2	199	0	100
(1)S*44*G1	200	0	100
G2	200	0	100
G3	200	0	100
G4	200	0	100
(1)S*46*G1	198	0	100
G2	198	0	100
G3	198	0	100
G4	198	0	100

Devices used to designate parental and hybrid isolates are explained in the text under Materials and Methods. All percentages have been calculated to the nearest whole number. Where less than 50 pairs of gametangia were counted the percentages only serve to indicate the approximate character of the isolate. Low counts usually occur in isolates where imbalance of the sexes is extreme and very few ♂–♀ pairs have been produced (cf. Table IV).

* The *javanicus* parent is bracketed because these putative hybrids, like all those designated by italics in Table II, revealed maternal characters only (see text).

opment (Controls, ♀s only) will be deferred until that subject is considered below.

Analysis of the first generation gametophytes showing intermediacy—TABLE II has been prepared in order to show how each cross was made and to list the key number of each derived sporophyte. As soon as gametophytes, (1)G, had been obtained from the first dozen crosses it became evident that two basically different types of genetic behavior were being exhibited. In one, indicated by italics in TABLE II, all of the derived gametophytes were pure H-type like the female parent of the cross. An interpretation and discussion of these crosses will be given later on. In the other, comprising the great majority of crosses made, many of the derived gametophytes showed a condition clearly intermediate between the two parents and proving beyond doubt that actual hybrids had been obtained. It is this group which will concern us more particularly.

Since, at first glance, it may seem surprising to find such striking intermediacy exhibited in haploid gametophytes, it is particularly important to understand the characteristics of these plants. Some of the primary pairs of male and female gametangia which they bear show the epigynous arrangement while others on the same hyphae show hypogyny. This intermediate condition will be designated by the letter I to distinguish it from the pure epigyny (E) or pure hypogyny (H) of the parents. Counts of paired gametangia from series of I-type gametophytes, grown on agar and all derived from a single hybrid sporophyte, revealed a whole scale of intermediacy ranging from nearly pure epigyny to nearly pure hypogyny. Representative counts illustrating this point are presented in TABLE III. It was clear, therefore, that the percentage of epigyny or hypogyny varied from plant to plant within the gametophytic F_1 population.

A second fact is also revealed in TABLE III. In addition to the intermediate strains obtained, both parental types reappeared when sufficiently large populations were analyzed. That is to say, some F_1 gametophytes showed only E-type gametangia and others showed only the H-type. While these plants were like their parents as far as epigyny and hypogyny are concerned, it would be erroneous to give the impression that they were like the parental strains in all ways. Actually gametophytes closely resembling the parental strains in all respects were extremely rare. Despite the relative structural simplicity of an *Allomyces* gametophyte, there is a fairly large number of observable characters which vary from strain to strain. Growth rate, presence of resistant sporangia (Emerson, 1941, p. 103), size and shape of gametangia, arrangement of gametangia

aside from epigyny and hypogyny (i.e., the occurrence of paired males or females and the occurrence of single males or females), intensity of pigment in the male cells, ratio of total male gametangia to total female gametangia, and other characteristics combined in manifold ways to give nearly every gametophyte of hybrid origin its own individual peculiarities. A detailed study of some of these features may prove to be worth while in the future. For the present the data in TABLE IV serve to illustrate the observations and show in particular how distorted the balance between the two sexes has become in certain strains.

TABLE IV

BALANCE OF THE SEXES IN PARENTAL AND SELECTED F1 GAMETOPHYTES

Isolates	Paired gametangia ♂+♂	♂+♀	♀+♀	All gametangia total	♂, %	♀, %
A. arbuscula (H)						
Burma *1A*	11	975	14	500	44	56
Ceylon *1*	82	917	1	500	54	46
Louisiana *1*	2	996	2	500	46	54
Philip. Isls. *1*	1	974	25	500	44	56
A. javanicus (E)						
Burma *1Da*	16	976	8	500	57	43
Burma *3*	41	959	0	500	53	47
Java *1*	39	943	18	1500	54	46
H × *E*						
(1)S31[1ZA4]G6	35	365	600	500	9	91
(1)S34[1ZB5]G3	205	420	375	500	32	68
(1)S79G2	312	105	583	500	40	60
(1)S55G54	314	255	431	500	42	58
(1)S106G5	172	573	255	500	48	52
(1)S55G2	268	608	124	500	55	45
(1)S103G2	171	805	24	500	57	43
(1)S68G1	948	34	18	500	92	8
(1)S54G1	943	52	5	500	95	5
(1)S75G3	999	1	0	2000	>99	<1

Bearing in mind that there had been much debate about the place of meiosis in *Euallomyces* (cf. Emerson, **1941**; Emerson and Wilson, **1949**) and that most of these genetic studies were made ten years before the cytological discovery of meiosis, one further point is of particular interest here. The experiments just described indicated that genetic segregation occurs in the resistant sporangia and that nuclei in the sporophytic mycelium are therefore diploid. Fortunately the asexual reproductive structures of *Allomyces* provided a precise means of proving this point more positively. The uninucleate diploid zoospores from

mitosporangia were used to obtain single-spore cultures of what we may term *secondary F_1 sporophytes*. Meiosporangia produced on these plants were germinated, and the spores therefrom were plated out. As shown in TABLE III (see S31[1Z] and S34[1Z]), the resulting population of gametophytes, from any given secondary sporophyte, exhibited the same range of segregation and types of gametangial arrangement as those revealed by the gametophytes derived from the primary F_1 sporophyte. Indeed this subculturing with single mitospores was carried through five successive asexual generations with exactly similar results (S35[5Z]). There could be only one interpretation of these findings: mitospores of *Euallomyces* are diploid, not haploid as Hatch (**1938**) had believed.

The sequence of steps involved in making and analyzing the interspecific crosses is outlined in FIG. 7. Before going on to the special problems presented by parthenogenesis and the F_2 and F_3 generations, it will be well to summarize the conclusions that could be drawn from the F_1 hybrids. In the first place, as has just been stated, there was clear proof of the diploidy of mitospores and strong indication of genetic segregation in the meiosporangia. This evidence, briefly reported over ten years ago (Emerson, **1941**), appears to have played a major role in Hatch's decision (**1944**) against his previous belief (Hatch, **1938**) that meiosis occurs at zygote germination. Recent cytological studies (Wilson, **1952**) have now proved beyond doubt that the meiotic divisions do indeed occur in the meiosporangia (i.e., resistant sporangia) of *Euallomyces*. In the second place it is very evident that the processes which determine the epigynous or hypogynous arrangement of gametangia, in the strains of *Euallomyces* used in the present work, are not controlled by a single pair of nonduplicated and regularly segregating alleles. Were a genetic mechanism of this sort involved, the haploid F_1 gametophytes should show a simple 1 : 1 segregation of E and H, and no intermediate types should occur. We might assume, therefore, either that gametangial arrangement is controlled by more than one pair of independently segregating alleles or that it is controlled by a single pair of alleles represented more than once in the F_1 sporophytic genotype and segregating independently. Some weight has been lent to the latter hypothesis by the discovery (see below) that the parental strains are polyploid and that many univalent chromosomes are distributed to the poles at random in the meiotic divisions of the F_1. In the third place, by studying the inheritance of the character, gametangial arrangement, we have at the same time gained a little insight into the mechanisms controlling sexual characters in *Euallomyces*. By making the E-type

by H-type cross, the well-ordered mechanism responsible for the regular formation of terminal or subterminal male gametangia in the primary pairs has been seriously interfered with in the resulting I-type gametophytes. Not only do E- and H-type pairs of gametangia occur on the same plant, but there is also frequently a great increase in the number of paired males and paired females as well as single males and females. Finally the fairly even balance between the total number of males and females on a particular plant is sometimes shifted far over in one direction or the other. All in all one gets the impression of a delicate, precise mechanism becoming badly out of adjustment. These maladjustments may ultimately lead us to a better understanding of the actual mechanism of sex determination in *Allomyces*.

Inheritance of gametangial arrangement in the F_2 and F_3—F_2 sporophytes were obtained simply by selfing the individual gametophytic strains derived from F_1 sporophytes and isolating the F_2 zygotes produced. F_2 gametophytes were then grown from meiospores in the usual way. Similarly, by carrying the cycle around again, F_3 gametophytes could be obtained. Analyses of the second and third generation plants revealed at once the general constancy of characters exhibited by all of the offspring of any given F_1 gametophyte. If, for example, as shown in TABLE V, intermediacy of gametangial arrangement characterized the F_1 strain, all the F_2 gametophytes derived from it would be intermediate also (e.g., (1)S31G3); if the F_1 showed pure epigyny, the F_2 would likewise be all E-type (e.g., (1)S42G5); and so forth. Moreover, just as each individual F_1 strain displayed its particular features of pigmentation, growth rate, balance of the sexes, etc., so its subsequent offspring would show essentially the same combination of characters.

These results may come as a surprise to those used to thinking in terms of the F_2 segregation exhibited by hybrids of higher plants and animals. We are of course dealing here with the haploid generation. Moreover it is most important to recognize the significance of the hermaphroditic, self-fertile condition in *Allomyces*. In contrast to the great majority of organisms that have been used in genetic work, producing the F_2 in *Allomyces* involves the most extreme sort of inbreeding possible. F_2 zygotes and the derived F_2 sporophytes are homozygous for all characters, and all meiospores will ordinarily receive identical sets of genes following chromosome reduction. Hence, the results just described are in precise conformity with our understanding of the life cycle of *Euallomyces*, with the genetic behavior of the F_1, and, as we shall see shortly, with what has been revealed by cytological studies of chromosome number and behavior in the parental and hybrid strains.

TABLE V

COMPARISON OF GAMETANGIAL ARRANGEMENT IN F1 GAMETOPHYTES AND THEIR F2 AND F3 PROGENY

Isolates	Pairs counted	E. %	H. %
(1)S31G3			
original	100	64	36
same plate	100	77	23
same plate	100	57	43
transf. x-center	100	44	56
transf. x-edge	100	46	54
transf. y-center	100	37	63
transf. y-edge	100	33	67
transf. x+1	100	52	48
transf. y+1	100	38	62
	Range	33–71	
	Av.	50	
(1)S31G3(2)S1G1	100	68	32
G2	100	78	22
G3	100	65	35
G4	100	66	34
G5	100	76	24
G6	100	68	32
G7	100	55	45
G8	100	74	26
G9	100	71	29
	Range	55–78	
	Av.	69	
(1)S42G3	62	26	74
(1)S42G3(2)S1G1 to G11	ca. 100 each		
	Range	20–90	
	Av.	58	
(1)S42G3(2)S2G1 to G10	100 each		
	Range	51–77	
	Av.	71	
(1)S42G5	144	100	0
(1)S42G5(2)S1G1 to G9	ca. 175 each		
	Range	99–100	
	Av.	100	
(1)S42G5(2)S2G1 to G10	ca. 175 each		
	Range	98–100	
	Av.	100	

Isolates	Pairs counted	E. %	H. %
(1)S49G1	121	0	100
(1)S49G1(2)S1G1	50	0	100
G2	50	0	100
G3	*50*	*40*	*60*
G4	100	0	100
G5	100	1	99
G6	100	5	95
G7	100	4	96
G8	100	2	98
G9	100	1	99
G10	100	0	100
(except G3)	Range	0–5	
	Av.	1	
(1)S51G7	45	85	15
(1)S51G7(2)S1G1	50	98	2
G2	50	96	4
(1)S51G7(2)S2G1	110	83	17
G2	73	78	22
G3	79	67	33
(1)S51G7(2)S1 G1(3)S1G1	100	92	8
(1)S53G5	149	100	0
(1)S53G5(2)S2*G1*	*50*	*50*	*50*
G2	*50*	*88*	*12*
G3	*100*	*50*	*50*
G4	*100*	*84*	*16*
G5	*100*	*70*	*30*
G6	*100*	*61*	*39*
G7	*100*	*66*	*34*
G8	*100*	*73*	*27*
	Range	*50–88*	
	Av.	*68*	
(1)S103G1	50	56	44
	50	44	56
	100	57	43
(1)S103G1(2)S2 G1(3)S1G1	100	64	36

Italics indicate those strains which appear to have mutated from the original F_1 (see text).

Detailed counts of epigyny and hypogyny in F_2 and F_3 populations derived from single F_1 gametophytes did reveal a considerable amount of variability in gametangial arrangement. These results suggested at first that the interpretation presented in the previous paragraph was incorrect and that some genetic segregation was occurring. It was established, however, by repeated counts made at various times on individual F_1 hybrid gametophytes which were propagated asexually by mycelial transfer, that an equal degree of variability occurred from one transfer to another and even from one region to another of the same culture. Representative figures are shown in TABLE V (see (1)S31G3). We believe that such variability can be explained by the relatively unstable condition that must exist following the disordering, by hybridization, of the normal mechanisms controlling the arrangement of gametangia and the balance of the sexes. No doubt the expression of these characters in the hybrids is subject, within the limits of the mixed genotype, to various subtle environmental factors none of which is yet understood. These observations do not, however, invalidate the conclusion that, with few exceptions, the F_2 and F_3 generations consistently and uniformly portray the general characters of the F_1 from which they were derived.

In contrast to the sort of fluctuations just described, which were apparently dependent upon environment and did not show sudden fixed changes, a particular hybrid strain would rarely exhibit what appeared to be a definite genetic mutation. Such changes, as shown by the examples in TABLE V (italics), usually involved a permanent shift from one category, E, I, or H, to another. Actually, the only three instances observed involved a change from E or H to I. While we do not yet have exact evidence of the specific genetic changes involved, it seems likely that such changes in gametangial arrangement result from irregular chromosome distribution which in turn may be correlated with the polyploid character of the parental strains (see below).

Viability of meiospores—Early in the genetic investigations it became apparent that the viability of meiospores from F_1 resistant sporangia of interspecific crosses was very much lower than that of meiospores from the parental strains. As knowledge was accumulated concerning methods which would permit mass germination of resistant sporangia, it was possible to determine meiospore viability with a fair degree of accuracy. Finally, with the detailed information presented by Wilson (**1952**) on the factors affecting maturation and germination of meiosporangia, precise viability tests became a routine matter in most instances. The procedures involved have already been outlined (cf. Methods, above).

Results of the viability studies are presented in TABLE VI, and the significant points revealed can be enumerated as follows: (1) Meiospores of the parental strains used in the present investigation have a generally high viability, usually ranging over 50% under the conditions of isolation, transfer, and plating employed. For reasons that are not yet fully understood certain strains of *Allomyces* obtained from nature show a much lower meiospore viability, but the use of such isolates with less than 50% viability was purposely avoided in selecting strains for crossing. (2) In sharp contrast with those of the parents, meiospores of the first hybrid generation are nearly always less than 5% viable, and not infrequently only one or two mature plants are obtained per thousand spores plated. It should be understood that a whole range of growth patterns can be recognized in such platings. A large majority of the spores fail to germinate at all. Quite a number produce feeble rhizoidal systems, sometimes with highly abortive, often swollen, short hyphal tubes, and ultimately die. A still smaller number develop into variously aborted plants which may reach considerable size but fail to form gametangia. And lastly, very few plants show essentially normal growth and reach full maturity. The plants in this category were the only ones counted in determinations of viability, and of course only when normal mature gametangia were produced could second and third generation progeny be obtained. This picture of grading lethality and abnormality in the F_1 population of gametophytes became completely understandable when it was correlated with the observations on chromosome behavior to be discussed below. (3) With a few exceptions, meiospores of the F_2 and F_3 generations exhibited a striking reversion to the high viability of the parents, and this could again be closely correlated with behavior of the meiotic chromosomes. The chart in FIG. 7 shows how the foregoing fluctuations in meiospore viability were related to chromosome behavior.

CHROMOSOME STUDIES OF THE ARTIFICIAL HYBRIDS

As has been repeatedly implied in our discussion of the interspecific crosses, cytological studies of meiotic nuclei in the parental and hybrid strains revealed a clear picture of the basic mechanisms responsible for the observed genetic behavior. Step by step correlation of the genetics and cytology evolved from two initial observations that were made early in the investigations of meiosis and have already been briefly reported (Emerson and Wilson, **1949**; Emerson, **1950**): the parental species have very different chromosome numbers; and meiosis in the F_1 resistant sporangia is highly irregular with only partial formation of bivalents and random distribution of many univalents.

TABLE VI

MEIOSPORE VIABILITY IN PARENTAL AND HYBRID ISOLATES

Isolates	Meiospores used: Spores/Plate	Total	% Viable
PARENTS:			
Burma *1A*	100	200	51.00
Burma *1Da*	50	200	44.00
Burma *3*	50	300	88.00
Ceylon *1*	50	200	76.00
Java *1*	100	300	52.00
Louisiana *1*	100	100	80.00
Philippine Islands *1*	100	200	60.00
			Av. 63.00
F1 HYBRIDS			
(1)S31–38	40 (each)	320	0.00
(1)S31–33 and 35–38	100 (each)	700	0.14
(1)S31–38	400 (each)	3200	0.15
(1)S31[1ZC1–5]	(various)	618	0.00
(1)S34[5Z]	1000	5000	0.10
(1)S38	1000	3000	0.15
(1)S103 (TYPE A)*	1000	4000	0.08
(1)S103	5000	10000	0.17**
(1)S110 (TYPE A)*	1000	5000	0.22
(1)S110	5000	10000	0.04**
(1)S110	5000	20000	0.21
			Av. 0.11
(1)S106 (TYPE B)*	40	40	2.50
(1)S106	100	100	6.00
(1)S106	1000	1000	2.50
(1)S106	5000	10000	ca. 2 00**
			Av. 3.25
F2 HYBRIDS			
(1)S32G4(2)S2	25	25	4.00
(1)S42G3(2)S1	15	15	27.00
S1	100	100	50.00+
S2	100	100	50.00+
(1)S42G5(2)S1	25	25	4.00
(1)S49G1(2)S1	25	25	71.00
(1)S51G3(2)S1	25	25	56.00
(1)S51G7(2)S1	25	25	4.00
(1)S53G1(2)S1	20	20	65.00
S2	20	20	45.00
S4	20	20	30.00
(1)S53G2(2)S1	25	25	56.00
(1)S53G5(2)S2	25	25	80.00
			Av. 41.70

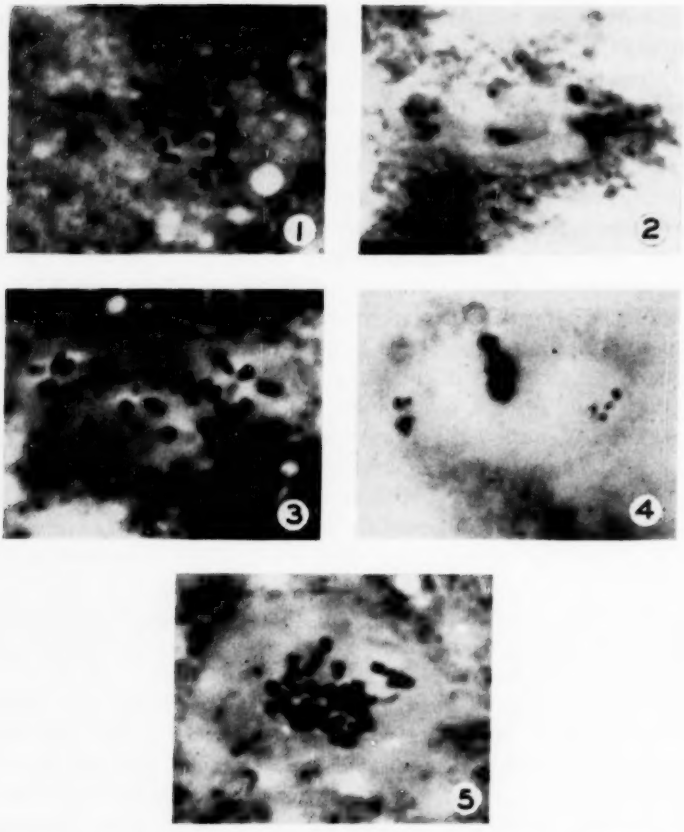
* The differences between Type A and Type B are discussed in the section on chromosome studies of the artificial hybrids.

** These data were obtained by Dr. John L. Ingraham.

To avoid confusion that might otherwise arise later on, two points require explanation before we proceed with this description of chromosome behavior. In the first place, it should be understood that most of the conclusions about chromosome behavior in the F_1 are based upon observations of hybrids 103–112. Crosses 1–102 were made over ten years ago, and the ample supplies of viable meiosporangia that had been preserved in a dry state provided a valuable source of F_1 gametophytes for selfing and further study. As Wilson (**1952**) has pointed out, however, meiosporangia dried for long periods seldom give sufficiently high and uniform germination to be used for studies of meiosis. Hence, although significant cytological observations were made on certain batches of dried material, detailed investigations of meiotic figures in the F_1 were all done with recent crosses that permitted mass germination of meiosporangia taken from fresh cultures.

In the second place it is important to note clearly the extent of our present knowledge about the chromosome numbers of the various parental strains used in the current hybridization studies. The information is provided in TABLE VIII, bold face entries. Emerson (**1950**, pp. 192–193) has already explained why the numbers first given (Emerson and Wilson, **1949**) for *A. arbuscula* (Ceylon *1*, *7* chromosomes) and *A. javanicus* var. *macrogynus* (Burma *1Da*, 14 chromosomes) were incorrect. Deferring for the present, then, other complications reported in the table, we can focus our attention on crosses 103–112, Ceylon *1*, with 16 haploid, by Burma *1Da*, with 28 haploid.

Type A and Type B nuclei in the F_1—With the same methods as those employed by Wilson (**1952**) to prepare and examine acetoorcein smears of germinating meiosporangia, it was found that two distinctly different patterns of chromosome behavior occur at Meiosis I of the F_1. In one, which we have designated "Type A," very little chromosome pairing occurs (cf. FIG. 1). At metaphase, one to five bivalents can be seen on the equatorial plate, and the univalents are distributed randomly to the poles, as shown in FIG. 2. In divisions of this type the chromosome number has been determined as 44, which is the sum of the two haploid sets received from the parents. The other condition existing at Meiosis I of the F_1 is characterized by many more bivalents and somewhat fewer univalents. This configuration, called "Type B," is shown in FIGS. 3 and 4. Because of the large number of chromosomes it was impossible to make exact counts, but careful study of seven nuclei revealed, respectively, 58, 51, 56, 53, 50, 58, and 59 chromosomes, giving an average of 55. From the approximate counts it is apparent that some doubling of the chromosomes must have taken place before germination



FIGS. 1–5. Meiosis I in interspecific hybrids of *Euallomyces: Allomyces arbuscula* × *A. javanicus* var. *macrogynus*. Photomicrographs, all approximately × 2660. 1. Type A, (1)S110. Prometaphase in an F_1 where the maternal chromosome set has not doubled; slight pairing is evident. 2. The same. A later stage in division where the univalents and some of the bivalents have reached the poles; other bivalents remain at the plate. 3. Type B, (1)S106. Prometaphase in an F_1 where the maternal chromosome set has doubled; there is much more pairing than in Type A (compare with FIG. 1). 4. The same. A later stage in division; the univalents are near the poles and the bivalents remain at the plate. 5. Prometaphase in an F_2, (1)S55G2(2)S2, with 44 bivalents. Pairing is regular because two identical sets of chromosomes were brought together in the zygote as a result of selfing (see text).

of the F_1 meiosporangia. Had the haploid complement of both parents doubled the total should approach $2\ (16 + 28) = 88$. Under these circumstances, moreover, a more complete pairing of chromosomes at Metaphase I might have been expected. If the haploid set of *A. javanicus* var. *macrogynus* alone had doubled, the count should be more nearly $2 \times 28 + 16 = 72$. If, on the other hand, only the chromosomes of *A. arbuscula* doubled, the total would be $2 \times 16 + 28 = 60$. This last number is reasonably close to the average of 55 actually determined. The degree of pairing and the total number of chromosomes suggest very strongly, therefore, that Type B nuclei do result from doubling of the haploid set contributed by *A. arbuscula*.

There is also good evidence that the doubling occurs prior to or during syngamy, before any mitotic divisions of the zygotic nucleus have taken place. Not only are Type A and Type B nuclear figures never found in the same meiosporangium, they never occur in different sporangia formed on one hybrid plant. In other words each F_1 hybrid exhibits the Type A behavior *or* the Type B behavior, never both. Nor is there any evidence of shifts from one type to the other. Hybrids that have been carried by repeated vegetative, mycelial transfers for more than 18 months continue to exhibit exactly the same chromosome pattern at Meiosis I. It seems clear that all the mitotically derived nuclei of a given interspecific hybrid are the same, and are either Type A or Type B.

A comparison of the reciprocal crosses lends further support to the foregoing interpretation of nuclear types. Of the 9 F_1 sporophytes which were examined cytologically (54, 103, 105–108, and 110–112), only those having *A. arbuscula* as the female parent showed Type B nuclei. No Type B's were found in crosses made with *A. javanicus* females. In the following section on parthenogenesis it will be shown that parthenogenetic formation of sporophytes, accompanied by a compensatory doubling of the chromosome number, is characteristic of certain strains of *A. arbuscula* but has not been observed in *A. javanicus*. The ability, therefore, to double the haploid set of chromosomes in female gametes of *A. arbuscula* is associated, on the one hand, with the apomictic formation of sporophytes when syngamy does not occur and, on the other hand, with the establishment of the Type B chromosome complement when syngamy is effected in interspecific crosses.

It must not be concluded, however, that the cross *A. arbuscula* ♀ × *A. javanicus* ♂ always gives Type B hybrids. Of the seven F_1 plants examined thus far, in all of which Ceylon *1* was the ♀ parent (54, 106–108, and 110–112), three are Type A (54, 110, and 112). Probably two main factors are involved in establishment of the nuclear condition: the

timing of gamete emergency and pairing, and the inherent hereditary tendency toward apomictic formation of sporophytes. When crosses are prepared it is not always possible to have gametes of the two parents being released from the gametangia at exactly the same time. If female gametes are subjected to males after some delay, the initial steps in parthenogenesis may already have occurred. The tendency toward apomixis is very slight in Ceylon *1* (cf. TABLES I and VII), and we could presume that Type A hybrids would be the rule when the female gametes are fertilized soon after their emergence. Type B would be more likely to occur when there is a delay before syngamy, during which time the tendency toward parthenogenetic development might lead to a doubling of the chromosome set in the female gamete. In Burma *1A*, apomictic formation of sporophytes occurs more frequently. This may account for the variable reaction of the gametes and the variable achievement of actual hybrids when Burma *1A* is the maternal parent. In such crosses, moreover, we would expect a high proportion of Type B even when syngamy follows emergence quite quickly. Unfortunately the dried resistant sporangia of hybrids made with Burma *1A* germinate too erratically to allow determination of their nuclear condition. Until, therefore, new crosses are made and careful attention is paid to the timing, the foregoing discussion of parthenogenetic tendencies and their relation to the frequency of Type B nuclei must remain largely theoretical.

Soon after the Type A and Type B hybrids had been discovered, we realized that the cytological differences between them should be reflected in their genetic behavior. If we were correct in concluding that the entire chromosome set of the *arbuscula* parent is doubled in the Type B, hypogyny should be more prevalent in gametophytes from Type B than in those from Type A. Making counts of gametangial arrangement is a painstaking, slow process which has limited the size of gametophytic populations which we could analyze.[3] The data presented in FIG. 6 are sufficient, however, to reveal the marked difference found between the A and the B hybrids. For reasons which we do not yet understand, epigyny is predominant in the populations of gametophytes from Type A. In Type B, on the other hand, as we had expected, hypogyny is clearly predominant. Hybrid S55 has not been examined cytologically, but its distribution of epigyny and hypogyny follows the pattern of Type A and indicates that the nature of any given hybrid, whether A or B, can be established genetically as well as cytologically.

[3] We take pleasure in extending a special expression of appreciation to Miss Ann Larson and Mrs. Dorothy Ellis Sistrom for their patient help in making these analyses.

Incomplete pairing and random chromosome distribution also account for the reduced viability of the F_1 meiospores which had been noted so many years before. When the two nuclear types in the hybrids had been discovered, more precise studies of meiospore viability were made and immediately revealed that spores from the Type B F_1 are, on the average, about thirty times more viable than those from Type A (see TABLE VI). While some difference in viability could have been pre-

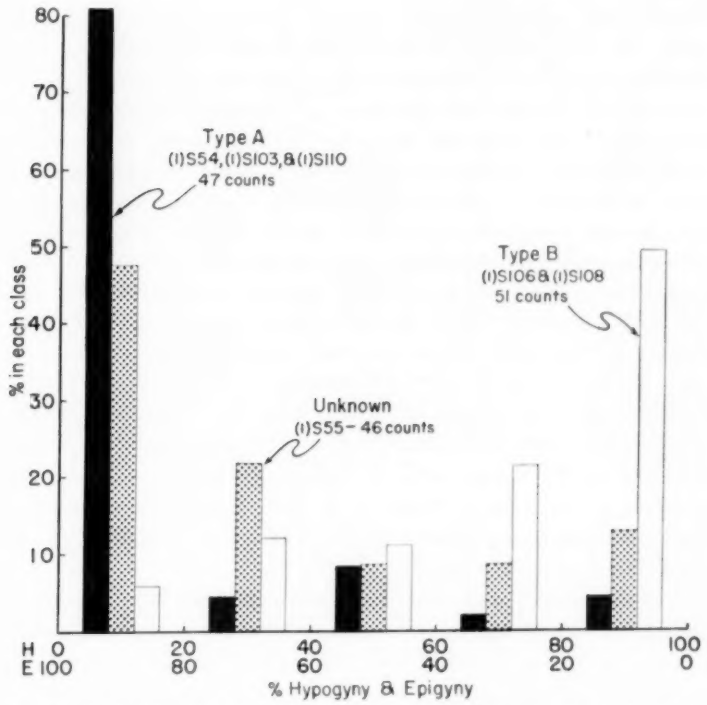


FIG. 6. Graph showing the proportions of epigyny and hypogyny in random populations of F_1 gametophytes from Type A and Type B hybrids of *Euallomyces*.

dicted simply from the greater amount of pairing and consequent more regular distribution of chromosomes in the Type B meiosis, such a marked difference supports the cytological evidence that the entire set of *arbuscula* chromosomes is doubled. With a full complement of chromosomes of one parent available to go to each pole at Meiosis I, viable genomes should occur much more frequently than if, as in Type A, nearly all of the chromosomes of both parental sets are distributed at random.

At present we can say little about the pairing of chromosomes in Type A nuclei. We do not know whether the bivalents are composed of homologous or partly homologous chromosomes, one from each parent, or whether pairing occurs between two chromosomes both from the same polyploid parent. The latter possibility is indicated by the polyploid character of the parental strains. This matter can be examined more fully following the report of chromosome numbers in the natural isolates (see below).

Chromosome behavior in the F_2 and F_3—The process and significance of selfing the F_1 gametophytes have already been described (cf. Inheritance in the F_2 and F_3). Bearing these points in mind, the main features of chromosome number and behavior in succeeding generations follow logically from the foregoing analysis of chromosome distribution at meiosis in the F_1. Whenever a meiospore nucleus contains a complement of chromosomes and genes sufficiently complete and balanced for normal growth, a mature gametophyte develops. Female gametes have precisely the same haploid chromosome set that the males have. Selfing results, therefore, simply in doubling this set to produce the diploid nucleus of the zygote. Quite understandably, meiosis in the F_2 and F_3 meiosporangia then proceeds in an orderly way as shown in FIG. 5, with the two identical sets of chromosomes usually exhibiting complete and normal pairing. The generally high viability of F_2 and F_3 meiospores (see TABLE VI) is a direct result of this regular chromosome behavior. Moreover, as could be predicted, the chromosome counts of the F_2 and F_3 progeny vary over a wide range, 20–44 pairs at meiosis in the material thus far examined (see TABLE VIII), because of random or partially random distribution at meiosis in the F_1.

No correlation has yet been made between the type of meiosis, A or B, in the F_1 and the range of chromosome numbers found in subsequent generations. The upper limit for both types would appear to be about 44 if the two sets of *arbuscula* chromosomes usually go to opposite poles in a Type B meiosis. The lower limit for progeny from Type B would, for the same reason, be about 16 in these crosses. The lower limit for Type A is less clear but might be as low as 8 considering the polyploid nature of the parents (see below).

Although many aspects of the inheritance of epigyny and hypogyny still remain obscure, it will be apparent that the general mechanism is revealed in broad outline by the foregoing observations on chromosome number and behavior. The various degrees of intermediacy can be accounted for by the variously balanced combinations of H chromosomes

from *A. arbuscula* and E chromosomes from *A. javanicus* var. *macrogynus* that occur as a result of haphazard chromosome distribution at meiosis in the F_1. Occasional recovery of pure E or H progeny is likewise understandable. Variation in other characters such as sex ratio and pigmentation can also be explained. That each individual F_1 gametophyte when selfed then ordinarily transmits its particular combination of gametangial arrangement and other characteristics uniformly

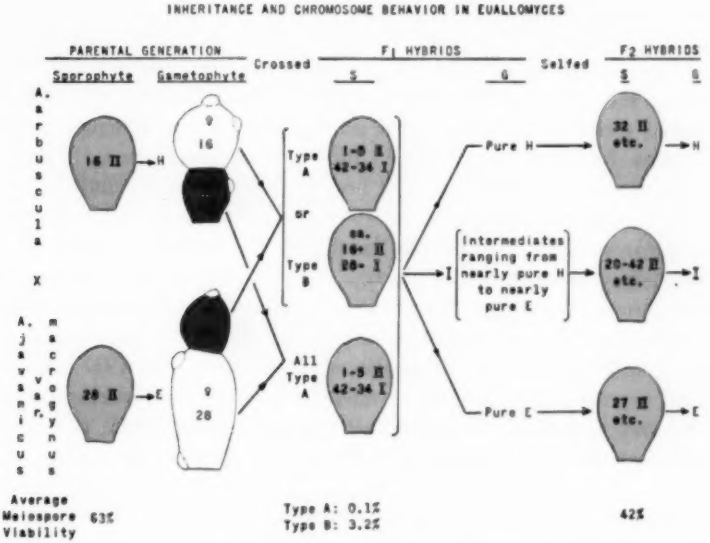


FIG. 7. Chart correlating the genetics and chromosome cytology of interspecific reciprocal hybrids in *Euallomyces*. Male gametangia are indicated by the orange color; female gametangia are colorless; meiosporangia are brown. The numbers within these structures show the chromosome counts and the extent of pairing at meiosis. The lettering is as follows: S, sporophyte; G, gametophyte; H, hypogyny; E, epigyny; A and B, the two types of nuclei in F_1 hybrids (see text).

to its offspring follows from the regular pairing of the identical chromosome sets at meiosis in the F_2 and subsequent generations. The chart shown in FIG. 7 is designed to summarize the main features of chromosome number and behavior in the artificial hybrids. Further discussion of the inheritance of epigyny and hypogyny, as well as an interpretation of the occasional sudden mutations that occur in the F_2 and F_3, will be presented in the following sections.

PARTHENOGENESIS

Before going on to the chromosome cytology of the natural isolates, it is necessary to consider briefly the question of parthenogenesis. The development of unfertilized female gametes has introduced into the genetics of *Allomyces* certain difficulties and complexities that could more happily be dealt with elsewhere as a separate problem. Yet these very complexities, as is so often true, have contributed a significant part in elucidating the over-all cytogenetic picture.

Interpretation of the first generation hybrids showing maternal characters only—It will be recalled that some 26 of the crosses, indicated by italics in TABLE II, gave gametophytes which were all like the female parent. Every one of the crosses in which female gametes of Philippine Islands *1* were used, and six out of nine of those in which female gametes of Burma *1A* were used, showed this type of inheritance. Parthenogenesis was not suspected at first because Kniep (**1930**) and Emerson, in the early studies leading to his 1941 paper, had both found that when female gametes of *A. javanicus* developed without fertilization, the resulting plants were all gametophytic. Since, therefore, the above crosses gave sporophytes, it was thought that fertilization and actual hybridization had been effected and that the peculiar pattern of inheritance must depend upon some sort of cytoplasmic dominance. In 1937, however, Sörgel reported that in two strains of *A. arbuscula* parthenogenetic plants may sometimes be sporophytic, and Emerson (**1941**, p. 99) then found that the phenomenon occurs quite frequently in several other strains of the species. Thus it became evident that apomixis must be carefully considered in interpreting genetic work with *Allomyces*.

Partly as a routine check on the procedures used to isolate male and female gametangia (see Methods), and partly to determine the behavior of the separated sexes, it was the usual practice in the course of each crossing experiment to plate out separately male and female gametes of the two parental strains. Sörgel (**1937**) and Emerson (**1941**) had established that ephebogenesis, the apomictic growth of male gametes, does not occur in *Allomyces*. Never, in the many control platings of male gametes, did any plants develop, so this possible complication can be entirely ruled out. TABLE I shows, however, the different patterns of behavior exhibited by segregated female gametes of the various parental strains. Although too limited to establish fully quantitative relations, the figures presented (Controls, ♀s only) indicate that sporophytes were never formed by *A. javanicus* (strains Java *1*, Burma *1Da*, and Burma *3*). Only very rarely were they produced by the Ceylon *1*

strain of *A. arbuscula*. They were, however, formed not infrequently by both the Burma *1A* and Philippine Islands *1* strains, the latter showing a somewhat greater tendency toward parthenogenetic formation of sporophytes than the former. Finally, a synthesis of the pertinent data from TABLES I and II reveals the interesting relations shown in TABLE VII. There is a strong positive correlation between (1) the parthenogenetic formation of sporophytes, (2) an atypical gametic reaction in the interspecific crosses, and (3) the production of putative hybrids with gametophytes showing maternal characters only. This evidence points clearly to the conclusion that the supposed crosses designated by italics in TABLES II and III are simply apomictic sporophytes bearing genetic material from the female gamete only.

TABLE VII

THE RELATION BETWEEN PARTHENOGENESIS AND "MATERNAL INHERITANCE"

♀ Parent	(1) Parthenogenetic formation of sporophytes	(2) Gamete-reaction in crosses	(3) "Hybrids" showing maternal characters		(4) Hybrids showing intermediacy	
A. arbuscula						
P.I. *1*	29%	Atypical	20/20	100%	0/20	0%
Burma *1A*	10%	Atyp.-Typ.	6/9	67%	3/9	33%
Ceylon *1*	<1%	Typical	0/47	0%	47/47	100%
A. javanicus						
(All)	0%	Typical	0/33	0%	33/33	100%

The question naturally arises, are apomictic sporophytes diploid or are they haploid? Sörgel (**1937**) concluded that they are diploid because the average volume of their nuclei appeared to agree closely with that of the nuclei in sporophytes derived from zygotes, whereas the haploid nuclei in gametophytic plants were about half as large. In the present investigation more conclusive evidence has come from direct cytological studies of the meiotic divisions in the meiosporangia formed by parthenogenetic sporophytes of Philippine Islands *1*. Prometaphase figures of Meiosis I (see FIG. 13) all show the normal diploid complement of 16 bivalents. We do not yet know precisely how the diploid condition is established in the apomicts, but probably it arises immediately following the first chromosomal division in the unfertilized female gamete by the formation of a nucleus containing the double haploid set. Further studies will be necessary to verify the exact position of the doubling as well as to reveal the effects of the presence of males or the influences exerted

by other environmental factors on the process of parthenogenesis in *Allomyces*.

CHROMOSOME NUMBERS IN THE NATURAL ISOLATES

Natural hybrids—Because of the fact that *Allomyces arbuscula* and *A. javanicus* var. *macrogynus* can be crossed in culture, and because they coexist in the same localities in nature, Emerson (**1941**) concluded that there was no reason to suppose that they did not cross in nature. He postulated that hybridization was a cause of intergradation between the species of the genus. The existence in nature of strains of *Allomyces* in which epigyny and hypogyny occur on the same individual plant has been known since Kniep (**1929**) first described *A. javanicus*. Emerson (**1941**) found several other natural isolates exhibiting this intermediate condition and designated them as var. *javanicus* to distinguish them from *A. javanicus* var. *macrogynus* which is characterized by 100% epigyny. The close morphological similarity between certain of the intermediate, I type, artificial hybrids described above and natural isolates of *A. javanicus* var. *javanicus* early suggested that this variety arose by natural hybridization between *A. arbuscula* and *A. javanicus* var. *macrogynus*. As we have seen, the artificial hybrids exhibit various haploid chromosome numbers ranging from 20 to 44. It was of particular interest, therefore, to determine the chromosome numbers of isolates of *A. javanicus* var. *javanicus* in order to discover whether all would have the same fixed number or whether, here too, there would be a range. Accurate counts have been made on only seven strains thus far, but they demonstrate conclusively that a range of chromosome numbers does exist here also, as shown in TABLE VIII. The chromosome counts in the artificial hybrids are, on the average, higher than those in the natural isolates, but the polyploid condition of both parents (see below) used in the laboratory crosses may well account for this difference. We believe there is ample evidence, from these chromosome numbers and the intermediate gametophytic characters, to conclude that the various strains of *A. javanicus* var. *javanicus* have indeed arisen in nature by a process of hybridization essentially similar to that which we have duplicated in the laboratory. Random distribution of the two sets of parental chromosomes at meiosis in the F_1 has resulted in various degrees of intermediacy of gametangial arrangement. Selfing in subsequent generations has stabilized this condition through normal pairing of identical chromosomes as shown in FIGS. 8 and 9, and meiospore viability (e.g., Java *1* in TABLE VI) is comparable to that of the parental species.

There seem to be no *a priori* reasons why all degrees of intermediacy in gametangial arrangement, ranging from nearly pure E to nearly pure H, should not occur in nature as they do in the laboratory crosses. It is a fact, however, that all of the 16 strains of *Euallomyces* isolated thus

TABLE VIII

SUMMARY OF CHROMOSOME COUNTS (HAPLOID NUMBERS) IN *ALLOMYCES* SUBGENUS *EUALLOMYCES*

Natural isolates	
A. arbuscula	
Brazil *2*	8
Portugal *1E*	8
Bali *1*	16
Burma 1A	16
Burma *1Db*	16
Ceylon 1	16
China *2B*	16
*Cuba *S33*	16
Fiji *B1*	16
*Fiji *H*	16
India *2*	16
Louisiana 1	16
North Carolina *2*	16
Philippine Isls. 1	16
*Queensland *1Ab*	16
*Trinidad *1A*	16
Belgian Congo *1*	24?
Queensland *1Aa*	22–26
Illinois *1*	32

Natural isolates cont'd	
A. javanicus var. *macrogynus*	
India *B4*	14
Burma 1Da	28
Burma 3 (original)	28
*Philippine Isls. *2*	28
Burma *3* (mutant)	50+
A. javanicus var. *javanicus*	
*Louisiana *2*	13
*Cuba *S30*	14
*Cuba *S55*	14 or 15
*Cuba *S4*	16 or 17
**India *B2*	19
Java 1	21
**Mexico *16*	21
Artifical hybrids	
A. arbuscula×*A. javanicus* var. *macrogynus*	
(1)S108G26(2)S1	22
(1)S103G19(2)S1	36
(1)S103G16(2)S1	42
(1)S103G18(2)S1	42+
(1)S55G2(2)S1	44
(1)S51G7(2)S1G1(3)S1	20
(1)S52G5(2)S4G1(3)S1	27
(1)S53G1(2)S2G1(3)S1	27
(1)S49G1(2)S1G1(3)S1	32
(1)S53G2(2)S1G1(3)S1	42

Bold face indicates the isolates used for crosses listed in Table II.

* These isolates were not listed by Emerson (1941). Further information about their origin and morphology will be published at a later date.

** Note that Mexico 16 and India B2 were included in *Brachyallomyces* by Emerson (1941). Subsequent discovery of their sexual stage has permitted final identification.

far and showing intermediacy are predominantly epigynous (mostly over 90%) and have, therefore, with one exception, been included in var. *javanicus* as originally defined (Emerson, 1941). Isolates showing a predominance of hypogyny may ultimately appear in natural collections,

but the data from artificial crosses suggest at least a partial explanation for the rarity of such forms. As we have explained previously (cf. FIG. 6), Type A hybrids are predominantly epigynous while Type B's show a preponderance of hypogyny. But Type B occurs only under special circumstances and may be very rare in nature. The prevalence of Type A, therefore, coupled with its predominant epigyny could account for the high frequency of epigyny in natural hybrids. Many other factors must be explored before the picture of gametangial arrangement in natural populations can be fully revealed.

The one exception, mentioned just above, of a predominantly epigynous intermediate which was not included in *A. javanicus* var. *javanicus* is Fiji *D2*. This natural isolate is so preponderantly male (96%) that it was given the varietal name *perandrus* (Emerson, **1941**). Similar segregants showing extreme shifts toward maleness, as well as others showing predominant femaleness (see TABLE IV), were also derived from the artificial crosses. This correlation of altered sex ratios in the laboratory hybrids with similar unbalance of the sexes in natural isolates further strengthens the conclusion that hybridization has been involved in the evolution of the varieties *javanicus* and *perandrus*.

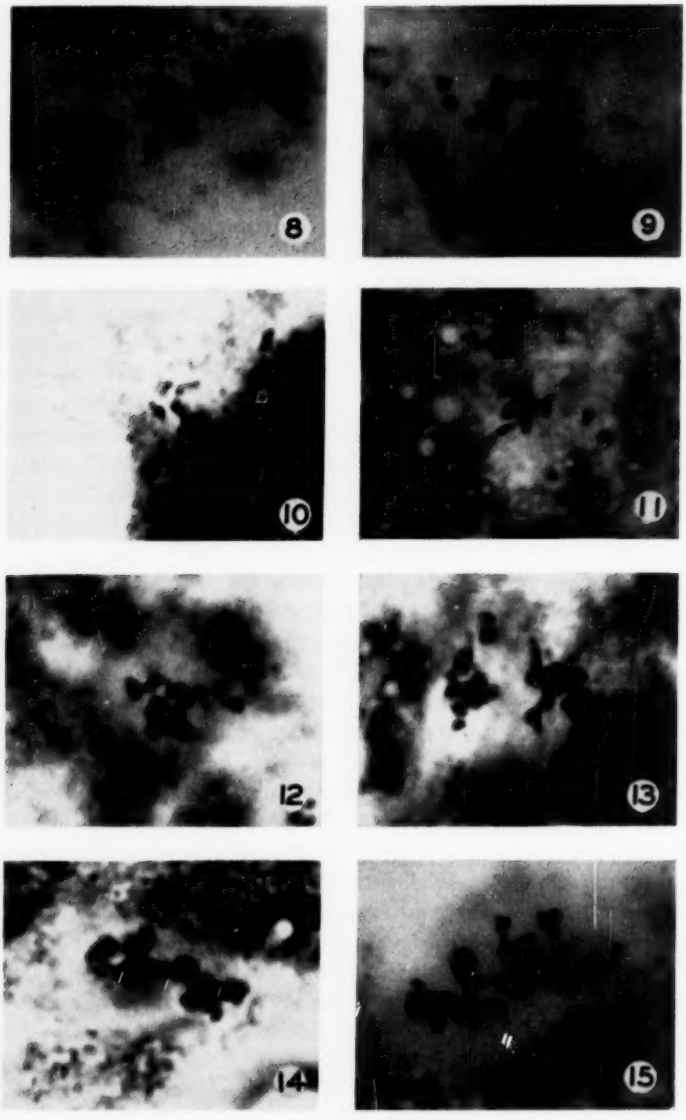
Although we have not yet consciously brought into our program of genetic work the interesting possibilities suggested by backcrosses, reference to TABLE II shows that crosses 19 to 28 were actually backcrosses. In other words, if our contention that var. *javanicus* is a hybrid is accepted, numbers 19–28 are backcrosses of a natural hybrid (*A. javanicus* var. *javanicus*, Java *1*) to a parental species (*A. arbuscula*, Ceylon *1*). Presumably Java *1* already has some *arbuscula* genes, and the progeny of Ceylon *1* × *Java 1* should show, on the average, more *arbuscula* characteristics than are exhibited by all other crosses (excluding Type B) where the epigynous parent was *A. javanicus* var. *macrogynus*. Time has not yet permitted us to make these detailed comparisons.

Polyploidy: its occurrence in nature and its significance in laboratory crosses—Not until most of the present cytological and genetic work had been completed was polyploidy discovered in the genus *Allomyces*. There were two reasons why this avenue of research was not explored in the early phases of cytological investigations. First, attention was focussed particularly on a limited number of isolates that were most easily handled in culture; strains, that is, in which meiosporangia germinated readily and the life cycle remained normal and stable. Second, polyploidy was simply not suspected. Although polyploids have been cytologically demonstrated in a few red and green algae (Drew, **1948**, and papers cited therein; Schussnig, **1950**), none, so far as we are aware, has been clearly established in any of the filamentous fungi.

As part of a general survey of chromosome numbers in *Allomyces*, about 24 isolates from various regions of the world were examined. These studies, first briefly reported by Wilson (**1952**), revealed a series of polyploids in *A. arbuscula* and the likelihood of a comparable series in *A. javanicus* var. *macrogynus*. The data presented in TABLE VIII indicate that the basic haploid number in *A. arbuscula* is 8 while that in *A. javanicus* var. *macrogynus* is 14. Further study will probably show that the number 22–26 is actually 24 and the number 50 + is 56. Thus, no definite aneuploids have been found, though there is no reason to suppose that they do not exist. Representative members of the polyploid series in *A. arbuscula* and *A. javanicus* var. *macrogynus* are shown in FIGS. 10–15.

The series in *A. javanicus* var. *macrogynus* is still open to question for several reasons. In the first place, this variety is relatively rare in nature. Only five or six isolates have been found as compared with about 60 of *A. arbuscula*. In the second place, of the small number of strains of var. *macrogynus* in our collection, only a few give the uniform mass germination of meiosporangia that is essential for studies of the meiotic chromosomes. In the third place, India *B4*, the isolate with 14 chromosomes, is exhibiting some variability and abnormality in the gametophytic phase so that some slight doubt remains as to its correct identity. And in the fourth place the high number, 50 +, is difficult to determine with certainty and does not, moreover, represent a natural condition so far as we know at present. The original strain of Burma *3*, used in all the early crosses, has 28 pairs of chromosomes at Meiosis 1 while the 50 + pairs occur only in a sub-strain which has arisen in the laboratory apparently by a doubling of the entire original chromosome set.

From this survey of chromosome numbers we have concluded that the strains of *A. arbuscula* used in the present crossing experiments are polyploid and that those of *A. javanicus* var. *macrogynus* are probably, but less clearly, also polyploid in origin. The apparent absence of detectable multivalent associations at meiosis in any of the parental strains, and the small amount of pairing in the Type A nuclei of the hybrids suggest that the polyploidy of the parents may not be of recent origin. There is, nevertheless, considerable likelihood that portions of each parental genome exist in duplicate in F_1 zygotes. These duplications may well explain the great range of variation that was found in the progeny of the artificial crosses and was dependent, as we have shown, upon the random distribution of chromosomes at meiosis in the F_1. Polyploidy, too, can account for the absence of definite ratios in the inheritance of the character gametangial arrangement and for the general difficulty encountered in attempting to interpret the results of the crosses



FIGS. 8–15. Prometaphase figures of Meiosis I in natural isolates of *Euallomyces*. Photomicrographs, all approximately × 2660. 8. Java 1, a natural hybrid with 21 bivalents. 9. Mexico 16, a natural hybrid with 21 bivalents. 10–13. Representatives of the polyploid series in *Allomyces arbuscula*. 10. Brazil 2, with 8 bivalents

prior to initiation of the cytological investigations. If, for example, the gametophytes of Ceylon *1* have two genes for H while those of Burma *1Da* have two for E, Type A sporophytes would be EEHH and random distribution and combination of these genes could readily explain the range from pure E to pure H exhibited by the F_1 gametophytes. According to our interpretation, Type B sporophytes, which show the same range but a preponderance of hypogyny, would be EEHHHHH.

One further point should be made before we conclude these theoretical considerations raised by the demonstration of polyploidy in *Allomyces*. In the discussion of inheritance in the F_2 and F_3 it has been stated that each of the hybrid strains usually continued to exhibit its particular characters, including gametangial arrangement, from generation to generation without marked change. This behavior is understandable in view of the sort of selfing that is possible in *Allomyces* and the regular chromosome pairing that ordinarily results at chromosome reduction in the F_2 and F_3. Evidence was also presented (TABLE V), however, of distinct, spontaneous changes that did occur occasionally. The polyploid condition of the parental strains provides a possible basis for explaining the gene changes that must be involved. It can be seen from the formation of some few bivalents at Meiosis I in Type A hybrids that the haploid sets of chromosomes of the parents include certain chromosome segments which are homologous. One member of any given pair may come from the chromosome set of one parent and one from the other. It is possible, however, that they both come from the same set and are partly homologous owing to their common origin at the time the polyploid condition first arose. In any event, whether they are from the same or different sets, since these homologous segments do not always pair they will sometimes be distributed as univalents to the same pole and hence will be included in the same daughter nucleus. If the meiospore containing this nucleus is viable and grows into a mature gametophyte, selfing will double the entire chromosome set and the particular homologous segment in question will be represented four times. In later meiotic divisions of the progeny these tetraploid portions

which are unusually small in this isolate. The outline of the small nucleus can just be discerned. 11. The same, showing the count of 8 more clearly. 12. Ceylon 1, with 16 bivalents. 13. Philippine Islands 1, with 16 bivalents. Two nuclei are shown in a parthenogenetically produced strain in which the normal chromosome number was restored by doubling of the maternal chromosome set in the female gamete (compare with FIG. 12 and see text section on parthenogenesis). 14–15. Representatives of the polyploid series in *A. javanicus* var. *macrogynus*. 14. India B4, with 14 bivalents. The chromosomes are unusually large in this isolate. 15. Burma *1Da*, with 28 bivalents.

of the sporophytic nuclei may cause irregularities in pairing which will result in occasional unpredictable genetic changes like those recorded in TABLE V. Certain irregularities, such as lagging chromosomes, have indeed been seen now and then at meiosis in the F_3. Hence, although the precise mechanism is not known, the occasional variability of F_2 and F_3 strains, which was long a disturbing factor in interpreting the genetic work, may be accounted for by the over-all cytological picture. The main pieces of evidence can be recapitulated as follows: (1) the two parental strains used are both polyploids; (2) they have different chromosome numbers; (3) pairing is variable and incomplete in the F_1; (4) random distribution of univalents results in a wide range of chromosome numbers and phenotypic characters in F_1 gametophytes; and (5) occasional irregularities in chromosome pairing in subsequent generations sometimes result in sudden fixed changes in phenotype.

TAXONOMY

By way of conclusion it is interesting to consider briefly what bearing the foregoing genetic and cytological studies may have on the established systematic groupings in *Allomyces,* more particularly the subgenus *Euallomyces.* In general, it can be said that the observations of chromosome number and behavior agree closely with and, hence, strengthen our present concepts of the species. This is particularly true of *A. arbuscula* in which hypogyny is associated with a basic haploid chromosome number of 8 and simple multiples to give 16, 24 and 32. The great majority of natural isolates of this species have 16 chromosomes in the gametophyte and 32 in the sporophyte and can, therefore, be considered as tetraploids. Emerson (**1941**, pp. 120 and 132) recognized that his division of *A. arbuscula* into the two varieties, *arbuscula* (with meiosporangia 32–45 μ wide) and *minor* (with meiosporangia 24–31 μ wide), represented an arbitrary separation made only for convenience. As could perhaps be expected, the only two 8-chromosome strains of *A. arbuscula* (Brazil *2* and Portugal *1E*) have small meiosporangia and fall within the limits of var. *minor.* It is clear, however, that no consistent correlation exists between ploidy and meiosporangium size since Louisiana *1* and Ceylon *1* (16 chromosomes) and Belgian Congo *1* (24 chromosomes) are also included in var. *minor,* and many 16-chromosome strains occur in var. *arbuscula.* If *A. arbuscula* is to be divided at all into varieties, a more significant basis might be the ploidy itself which would give us four distinct groups with haploid numbers of 8, 16, 24 and 32, respectively.

The isolates now included in the species *A. javanicus* present a rather

more complicated picture, and here the genetic and cytological observations certainly clarify matters greatly. There is convincing evidence that the strains which constitute *A. javanicus* var. *javanicus*, including Kniep's type culture, Java *1*, arose as hybrids. A natural corollary to this conclusion is the fact that *A. javanicus* var. *macrogynus*, with its pure epigyny, its basic haploid chromosome number of 14, and its probable polyploid series 14–28–56, must be recognized as a distinct species. We are proposing the following nomenclatural scheme, therefore, as one that best fits the facts, that shows the natural relationships within the species, and that is in conformity with present international rulings.

Allomyces macrogynus (Emerson) comb. nov.
A. javanicus var. *macrogynus* Emerson (**1941**)
? *A. javanicus* var. *japonensis* Indoh (**1940**)
Allomyces × **javanicus** (Kniep) stat. nov.
A. javanicus Kniep (**1929**)
A. javanicus var. *javanicus* Emerson (**1941**)
A. javanicus var. *perandrus* Emerson (**1941**)

It might be thought that Indoh's varietal name *japonensis* should more properly be used for the new combination. Our reasons, however, for retaining *macrogynus* are the following. In his doctorate thesis, completed in 1937, Emerson described *A. macrogyna* as a new species and this name appeared in the summary of theses from Harvard University published in 1938. The name *A. macrogyna* was again used by Emerson and Fox (**1940**), but in 1941 Emerson, in giving the Latin diagnosis, adopted a more conservative treatment by designating *macrogynus* as a variety of *A. javanicus*. At that time, Emerson (*loc. cit.*, ff. p. 133) pointed out the close similarity between his var. *macrogynus* and var. *japonensis* Indoh (**1940**) but suggested that both names be retained pending a detailed comparison of living specimens. This comparison has not yet been made and will only be possible if viable material of Indoh's isolate still exists. Because of the slight doubt that remains regarding the identity of the two entities, and because the name *macrogynus* has been used consistently throughout the work of the past decade, we are retaining *macrogynus* as an epithet which describes one of the distinctive morphological features of this species.

Only one of the several isolates in the subgenus *Cystogenes* has given sufficiently good germination of meiosporangia to permit establishment of the chromosome number. Wilson (**1952**) found 14 bivalents at Meiosis I in Burma *1B*. Studies of other isolates are being continued

in an effort to determine whether polyploid series occur here, as in *Euallomyces,* and whether or not *A. cystogenus* and *A. moniliformis* have the same chromosome number.

Chromosome counts in *Euallomyces* have revealed no correlation between ploidy and geographical distribution. This is not surprising in view of the world-wide occurrence of all species of *Allomyces.*

DISCUSSION

Because the various ideas and conclusions relating to each of the several aspects of the foregoing report have already been set forth in some detail, only a few general comments remain to be made. One of the great chapters of plant science and biology that has been written during the past half century is based upon the synthesis of taxonomy, genetics and cytology. Knowledge of the phylogenetic relations of higher plants and insight into many of the basic processes of evolution have been gained by an impressive array of fundamental contributions in these combined fields of study. Despite unique advantages which the thallophytes offer for such investigations, they have thus far played a relatively minor role. Many of them, like the marine algae as well as certain fresh water forms, are difficult to culture, while the small nuclei of the fungi have for the most part long defied any detailed studies of chromosome behavior. Increased understanding of cultural requirements and modern cytological techniques are, however, rapidly removing these obstacles. The work with *Allomyces* is still fragmentary, but even in this preliminary phase it has shown what opportunities exist. For the first time it has been possible to use a microorganism that can be grown in culture under controlled conditions for a combined study of taxonomy, natural and artificial hybridization, polyploidy, and chromosome behavior.

We have previously stressed (Emerson and Wilson, **1949**) those features of the life cycle and development of *Euallomyces* that make it specially suited to various kinds of basic biological research. Particularly noteworthy are the independent haploid and diploid phases, the self fertile hermaphroditic sexual mechanism, the marked differentiation of sexual cells, and the precision with which each part and process of the life history can be controlled at will. Strains of *Allomyces* of known chromosome number and tested behavior are currently being tried out in several laboratories in the United States where gene action, radiation effects, and other problems are being studied. Like all living things *Allomyces* has its special quirks and peculiarities with which the investigator must be as familiar as possible if he is to interpret his experiments

correctly and take maximum advantage of the possibilities offered. The apomictic formation of sporophytes, the brachy-cycle, the occurrence of resistant sporangia on gametophytes—these deviations from the ideal life cycle (Emerson, **1941**; Wilson, **1952**), as well as other characteristics stressed in the present paper, must always be kept clearly in mind.

Of the many avenues of research with *Allomyces* that have been opened up by the combined cytogenetic and cytotaxonomic approach, three deserve special mention because they lead so directly from the findings reported herein. (1) The inheritance of gametangial arrangement can now be investigated, without the complication of polyploidy, by crosses of parental strains each having the basic chromosome number, i.e., *arbuscula* (8) × *macrogynus* (14). (2) The pairing and distribution of chromosomes in *intra*specific hybrids within each polyploid series may indicate the degree of homology that exists among chromosomes of the various sets and give insight into the evolution of the species. (3) Having demonstrated the presence of polyploidy in all strains of *Allomyces* previously used for studies of induced mutation, we can understand the rarity of mutants and turn our attention to isolates with basic chromosome numbers. Detailed investigations of the cultural requirements and metabolism of *Allomyces*, presently being completed in our laboratory, will round out the fundamental background and enable us to employ this phycomycete for studies of biochemical genetics and gene effects in a life cycle composed of separate haploid and diploid generations.

SUMMARY

This paper presents a report of genetic, cytogenetic, and cytotaxonomic studies made with the aquatic fungus *Allomyces* over the past seventeen years. Because there have not, hitherto, been any extensive genetic analyses of interspecific hybrids in the Phycomycetes, the isolates and procedures used in these investigations are described in some detail. The life cycle and structural characters of the two species of *Euallomyces*, *A. arbuscula* with hypogynous male gametangia, and *A. javanicus* with epigynous male gametangia, are reviewed, and emphasis is placed on those features that are of special significance in genetic work. Methods for segregating the gametes, making the crosses, isolating the hybrid zygotes, determining viability of the products of meiosis (meiospores), and analyzing the progeny are explained.

Reciprocal matings made between four strains of *A. arbuscula* and three of *A. javanicus* in various combinations show that the reaction of the gametes and the percentage of "takes" vary according to the par-

ticular parents used. Counts of epigyny (E) and hypogyny (H) in the gametophytes derived from over a hundred F_1 sporophytes have demonstrated that, in addition to both the parental types (E and H), a whole series of intermediate strains (I) segregate out. From these results it is concluded that the arrangement of gametangia is controlled either by more than one pair of independently segregating alleles or by a single pair of duplicated alleles that segregate independently. The viability of meiospores from F_1 sporophytes is shown to be reduced to less than one tenth that of the parents.

In about one-fifth of the crosses *all* of the progeny exhibited pure hypogyny (H). For the following reasons we believe that hybridization failed to occur in these instances, the "F_1" sporophyte having arisen by parthenogenetic development of the female gamete. (1) The female parent was always *A. arbuscula.* (2) Apomictic formation of sporophytes, accompanied by chromosome doubling, characterizes certain strains of *A. arbuscula,* and it was from "crosses" of just such strains that the purely H-type progeny arose. (3) The reaction of the gametes in these crosses was atypical and zygotes were not seen.

Second and third generation progeny from the true crosses generally exhibit high meiospore viability and perpetuation of the characters (E, H, or I) of the F_1 gametophytes from which they were derived.

Studies of chromosome number and behavior in parental and hybrid strains have provided a precise basis for understanding and interpreting the genetic experiments just summarized. A polyploid series consisting of natural strains with 8, 16, 24, and 32 haploid chromosomes exists in *A. arbuscula,* and counts of 14, 28, and 50 + (probably 56) have been established in *A. javanicus* var. *macrogynus.* The parental strains originally selected for hybridization have 16 and 28 chromosomes respectively in their gametophytic nuclei, and hence the sporophytes, with 32 and 56 chromosomes, represent a tetraploid condition.

Two patterns of chromosome number and behavior have been observed at the first meiosis in F_1 hybrids obtained by crossing these tetraploid parents. In Type A nuclei, there are $16 + 28 = 44$ chromosomes, but only very few bivalents, and the univalents are distributed randomly to the poles. In Type B nuclei approximately 55 chromosomes have been detected; about half of them form bivalents, and again many univalents go to the poles at random. The irregular distribution of chromosomes in both types accounts for the greatly reduced viability of F_1 meiospores. More precise determinations within the hybrid population show, furthermore, a direct correlation between the degree of pairing and the percent meiospore viability: Type A, few pairs and ca. 0.1%

viability; Type B, many pairs and ca. 3% viability. From the observed number of chromosomes, the amount of pairing, and the fact that Type B hybrids always have *A. arbuscula* as the female parent, it is concluded that Type B nuclei arise by a doubling of the *arbuscula* complement of chromosomes during or just prior to syngamy, i.e., $2 \times 16 + 28 = 60$ (55 detected).

The irregular distribution of univalents in the F_1 explains the variable haploid chromosome numbers (20–44) which are found to occur in the F_2 and F_3 progeny and also gives insight into the mechanism whereby E and H types, as well as a series of intermediates, I, segregate out. Chromosome pairing is usually regular and complete again in F_2 and F_3 meioses, and meiospore viability returns to the high level of the parents.

All of the evidence, morphological, genetic, and cytological, points to the conclusion that *Allomyces javanicus* var. *javanicus* is a natural hybrid of *A. arbuscula* × *A. javanicus* var. *macrogynus*. Gametophytes are characterized by an intermediate condition in which epigyny and hypogyny occur on the same plant; this intermediacy has been exactly duplicated by crosses made in the laboratory; and the chromosome numbers of natural isolates range from 13 to 21, just as those of the F_2 and F_3 from artificial crosses vary, though over a somewhat higher and wider range. We are therefore raising var. *macrogynus* to a species, *A. macrogynus* n. comb., and designating *A.* × *javanicus* Kniep (*arbuscula* × *macrogynus*) as a hybrid species including var. *javanicus* and var. *perandrus*.

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THE EFFECT OF TEMPERATURE AND NUTRIENTS UPON SPORE GERMINATION OF THE OAK WILT FUNGUS [1]

CHARLES L. FERGUS

The method by which long-distance overland spread of oak wilt is accomplished in nature remains unknown, but the discovery that *Endoconidiophora fagacearum* Bretz produces mycelial mats which rupture the bark and expose large numbers of endoconidia and ascospores indicates the potential ability of the fungus to produce abundant inoculum suitably located for natural spread. Both spore forms have been used by various workers to induce wilt artificially in oak trees. Henry and Riker (**5**) concluded that wounds serve as infection courts and in all later artificial inoculation work (**1, 2, 3**) wounding has been employed. In natural infection, if spores land on a wound or if they are introduced into the tree through the wounding activities of some unknown agent, they must be capable of germinating there to establish the disease relationship.

In order to assist in the evaluation of the suspected role of the spores in long-distance overland natural spread several aspects of germination were studied. The results are reported herein.

MATERIALS AND METHODS

Endoconidia were obtained by incubating isolate *127* (obtained from a wilting red oak) on Nutramigen [2] agar slants (16 gm Nutramigen and 17 gm agar dissolved in one liter distilled water) for two weeks at 22° C. The endoconidia were washed from the surface of the slant with sterile distilled water and adjusted to a concentration of 100,000 spores per ml using a Fuchs-Rosenthal counting chamber.

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[2] Nutramigen is a baby food produced by Mead Johnson and Co., Evansville, Ind. Its use as a nutrient medium was suggested to the author by Dr. G. H. Hepting and Dr. V. M. Cutter, Jr. The oak wilt fungus sporulated more readily and more abundantly on this medium than on any other tried.

In the experiment studying the effect of nutrients on germination the endoconidia were washed three times in sterile distilled water by centrifugation and decantation.

Perithecia were obtained by spermatizing isolate *127* (compatibility A) with *78* (compatibility B). The ascospores were harvested one week after spermatization under a dissecting microscope, using sterilized needles to pick the sticky ascospore mass off the beaks of the perithecia. Extreme care was taken to be sure that only ascospores were obtained. The masses were then placed in a small amount of sterile distilled water in a short test tube of small diameter and agitated and crushed with a

TABLE I

THE EFFECT OF TIME AND TEMPERATURE ON GERMINATION OF ENDOCONIDIA OF E. FAGACEARUM IN DISTILLED WATER DROPS ON MALT EXTRACT AGAR

Temperature °C	Percent germination after				
	16 hrs	24 hrs	40 hrs	64 hrs	88 hrs
3	0	0	30	70	74
6	0	0	46	90	94
9	1	16	66	88	98
12	24	36	80	98	S[a]
15	50	56	90	S	
18	80	98 S	S		
21	84	100 S	S		
22	94	100 S	S		
24	96	100 S	S		
28	98	100 S	S		
31	96	100	100	100	100
32	96	100	100	100	100
33	22	20	22	28	32
36	0	0	0	0	0
40	0	0	0	0	0

[a] S indicates abundant production of endoconidia from the germ tubes.

sterile glass rod to free the spores from the mass. Although the ascospores tend to remain sticking together, this method effectively loosened them so that a homogeneous mixture of individual spores was finally obtained. The ascospore suspension was adjusted to 100,000 spores per ml.

Preliminary experiments had shown that spores germinating in a liquid quickly produce "secondary" endoconidia from the germ tubes, making it difficult to attach numerical significance to the results. Even on agar surfaces the germ tubes form secondary endoconidia, but their formation is slower depending upon the amount of surface moisture present. Therefore the hanging drop method was not used in these

studies; instead one drop of the spore suspension was pipetted to each of two spots on agar media poured several days earlier in petri dishes.

Observations on spore germination were made at various time intervals by counting under high power of the compound microscope the number of germinated and non-germinated spores in two fields of each of the two drops. All of the spores in the field were counted. The results are presented as percent germinated spores. A spore was considered to have germinated only if a germ tube were recognizable.

The agar media on which the spores were germinated varied in the different experiments. Malt extract agar (17 gm malt extract, 17 gm agar per liter) was used in the temperature experiments. In the experiments studying the effect of nutrients, decoctions, and infusions, plain agar (2 percent) was used.

To make the infusions, 50 gm (fresh weight) of material were cut into small pieces and steeped in 250 ml distilled water for 24 hours at room temperature. The infusion was filtered through cheesecloth and distilled water was added to the filtrate to make a final volume of 250 ml. The decoctions were made in a similar manner except that the material was boiled for 30 minutes. The infusions and decoctions were sterilized by filtration through a Seitz filter. The material used was healthy living red oak wood and bark and dead red oak wood and bark.

EXPERIMENTAL RESULTS

The results of germination at different temperatures (TABLES I, II) show that endoconidia and ascospores germinate over a wide temperature range. The temperature at which the maximum number of spores germinated in the shortest time was 28°. However, at temperatures below and above this point germination was abundant, although slower. There appear to be no significant differences between the germinability of the endoconidia and ascospores, although the latter do appear to require more time to germinate at some temperatures than do the endoconidia. The minimum temperature was not found, since the spores germinated at 3° C, the lowest temperature tried. The maximum temperature is between 33° and 36° C and the optimum is a range from 21° to 32° C. The time required for the spores to germinate, the rate of growth of germ tubes and the production of secondary endoconidia by the germ tubes were affected by the temperature. For example, after 170 hours at 6° C the average length of germ tubes of 50 germinated spores was 38 microns and no secondary conidia had been formed. After 64 hours at 31° C the average length of germ tubes was 35 microns and no secondary conidia had formed.

Interesting morphological changes accompany spore germination at the different temperatures. When the endoconidia are placed in a liquid they begin to swell rapidly, attaining a size three to four times larger than their original size. The shape changes with the spore gradually assuming a globose form. This is usually regular but sometimes the swollen spores are irregularly globular. Usually only one germ tube forms from each spore, but occasionally two tubes develop. At high temperatures the germ tubes branch earlier than at the lower temperatures. At more optimum temperatures germ tubes begin to form endogenous spores within 16 hours. At first these are easily distinguished from the spores originally placed in the solution because of the differences

TABLE II

THE EFFECT OF TIME AND TEMPERATURE ON GERMINATION OF ASCOSPORES OF E. FAGACEARUM IN DISTILLED WATER DROPS ON MALT EXTRACT AGAR

Temperature °C	Percent germination after				
	16 hrs	24 hrs	40 hrs	64 hrs	88 hrs
3	0	0	24	52	70
6	0	0	30	80	83
9	0	20	78	86	94
12	12	50	86	96	96
15	64	70	90	S[a]	
18	62	92	S		
21	82	92 S	S		
22	70	96 S	S		
24	92	96 S	S		
28	98	100 S	S		
31	58	72	96	96	98
32	66	82	90	90	92
33	12	14	14	14	18
36	0	0	0	0	0
40	0	0	0	0	0

[a] S indicates abundant production of endoconidia from the germ tubes.

in size and shape. However, these secondary spores soon begin to swell and assume a globose shape. High and low temperatures inhibit secondary spore formation even though they may not inhibit germ tube growth. Germ tubes as long as 118 microns with extensive branching have been observed with no endospore formation. At more optimum temperatures endospores have been observed forming in germ tubes 36 microns long.

Ascospores also swell rapidly when placed in solution, attaining two or three times their normal size. In the process of swelling the ascospore loses its curved shape, appearing spindle-like, eventually becoming

elliptical. Rarely the ends do not swell equally. The germ tubes usually arise from the sides of the ascospores and usually only one is produced, although two have been observed occasionally. The germ tubes from ascospores also produce secondary endoconidia.

Although germination occurred above 31° C growth did not continue at these high temperatures and no secondary endospores were formed by the germ tubes. Microscopic examination after 170 hours at 32° C revealed that the contents of spores and germ tubes appeared quite granular, yellowish and with indistinct walls. At 36° C the spores appeared plasmolyzed. This indicated the possibility that the spores had been killed by the high temperature. Ascospores and endoconidia were exposed to 36° C (97° F) and 40° C (104° F) for various time intervals

TABLE III

PERCENT GERMINATION OF E. FAGACEARUM IN DIFFERENT NUTRIENT CONDITIONS AT 24°C AFTER 16 HOURS

Medium	Percent germination	
	Endoconidia	Ascospores
Sucrose 4%	49	44
Sucrose 2%	90	30
Sucrose 1%	94	46
Glucose 4%	68	58
Glucose 2%	80	36
Glucose 1%	100	64
Yeast extract 0.1%	100	90
Mineral solution[a]	42	32
Rain water	34	36
Distilled water	44	38

[a] The mineral solution was NH_4NO_3, 3 gm; $MgSO_4$ $7H_2O$, 2.22 gm; KH_2PO_4, 2.59 gm; K_2HPO_4, 2.21 gm; distilled water, 1 liter; (pH 6.5).

on malt agar plates. The plates were returned to 24° C and after 16 hours the per cent germination was determined.

Exposure to 36° C for 34 hours and to 40° C for 24 hours killed all the endoconidia and ascospores. Exposures for shorter times at both temperatures did not kill all the spores, e.g. after 12 hours exposure at 36° C, 100 percent of the endoconidia and 76 percent of the ascospores germinated. After 12 hours exposure to 40° C, 50 percent of the endoconidia and 28 percent of the ascospores germinated.

Germination in different nutrient conditions was studied to determine whether the spores require an external food or mineral source in the environment for germination (TABLE III).

The spores germinated best when an organic food substance was present. Yeast extract and 1 percent glucose were the best media.

However, the spores germinated well in both rain water and distilled water.

Although the method by which the oak wilt fungus gains entrance into a tree is still unknown, one suspected avenue is through wounds. In the moisture which might accrue or be deposited in such wounds, various chemicals from the tree would diffuse and go into solution. To determine whether such substances are stimulatory or inhibitory to spore germination, various kinds of oak decoctions and infusions were made. Mixtures of the infusions and decoctions were also tested. The original concentrations of the infusions and decoctions were diluted to one-half, one-fifth and one-tenth.

The endoconidia and ascospores germinated well in these solutions. Best germination (98 percent) occurred in a mixture of infusions of dead bark and healthy wood. Germination was better in the more dilute concentrations. A two-fold increase in percent germination over germination in distilled water was observed in many of the solutions.

DISCUSSION

The results of endoconidial germination obtained agreed in general with those of Young (**6**) who found that maximum spore germination occurred at 25° to 30° C on 1 percent dextrose agar. Henry (**4**) observed maximum germination at 24° C, but his results are not exactly comparable to those reported herein since the endoconidia were germinated in a liquid.

The effect of temperature upon secondary endospore formation by conidial germ tubes reported here agrees with the observations of Young (**6**).

From the results of these laboratory studies it is probable that the endoconidia and ascospores of *E. fagacearum* will germinate if deposited in a moist wound of a tree at normal temperatures. Some of the concentrations of decoctions and infusions were probably greater than those which the spores would encounter in nature, yet germination was abundant indicating that no toxic materials were present.

Although exposure to high temperatures killed ascospores and endoconidia, the length of exposure necessary was greater than would prevail in nature in the summer, since such temperatures occur for only short periods of time.

It appears logical to conclude that both endoconidia and ascospores may serve as inoculum for overland spread of oak wilt, since they can germinate under a variety of conditions, although the exact method of their distribution remains unknown.

SUMMARY

The optimum temperature for the germination of ascospores and endoconidia of *E. fagacearum* is within the range of 21° to 32° C; the maximum is between 33° and 36° C; and the minimum is below 3° C. The time required for the spores to germinate, the rate of growth of germ tubes and the production of secondary endoconidia were affected by temperature. Exposure to 36° C for 34 hours and to 40° C for 24 hours killed the endoconidia and ascospores.

Sucrose, glucose and yeast extract permitted high percentage germination; however, the spores germinated fairly well in rain water and distilled water. Decoctions and infusions of living and dead red oak bark and wood and various mixtures of these solutions allowed increased germination over germination in distilled water.

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THE INFLUENCE OF CONCENTRATED SUBSTRATES ON THE DIAMETER ATTAINED BY THE FUNGUS COLONY [1, 2]

F. P. Brancato [3] and N. S. Golding

(with 5 figures)

The references in the literature to the tolerance of molds to highly concentrated media are many. In contrast, the relationship of progressively more concentrated substrates to the growth of molds has been incompletely studied. Rarely has it been studied with reference to the altered rate of increase in diameter of the colony (Golding, **1926**; Galloway, **1935**).

In a preliminary study certain common molds showed a marked initial increase in diameter when glucose was added to a medium commonly accepted as adequate. This stimulation generally reached a maximum near 0.55 molal concentration of glucose and then declined proportionately with increase in the sugar concentration. It was felt that some of the possible causes of this phenomenon should be investigated further. The following likely explanations were considered most important and were studied individually:

1. The amount of oxygen in solution was reduced to a level more favorable for growth.
2. The additional glucose served as an added source of nutrients.
3. The oxidation-reduction potential of the medium was altered to a more favorable level.
4. The pH of the basal medium was changed to a more favorable level.
5. Growth was influenced by osmotic pressure changes.

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[2] This investigation was supported in part by funds provided for biological and medical research by the State of Washington, Initiative Measure 171.

[3] Data from thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bacteriology, Department of Bacteriology and Public Health, The State College of Washington. Present address is the United States Public Health Service Hospital, Seattle, Washington.

The possibility exists, however, that growth was influenced by all or by some combination of these factors.

MATERIALS AND METHODS

The same strains of *Aspergillus niger, A. flavus, Penicillium notatum, P. expansum, P. roqueforti,* and *Geotrichum candidum* used in a previous study (Brancato and Golding, **1953**) served as the test molds. The preparation and inoculation of the basal malt agar (Difco) medium and the method of recording growth are also described in this paper. Both

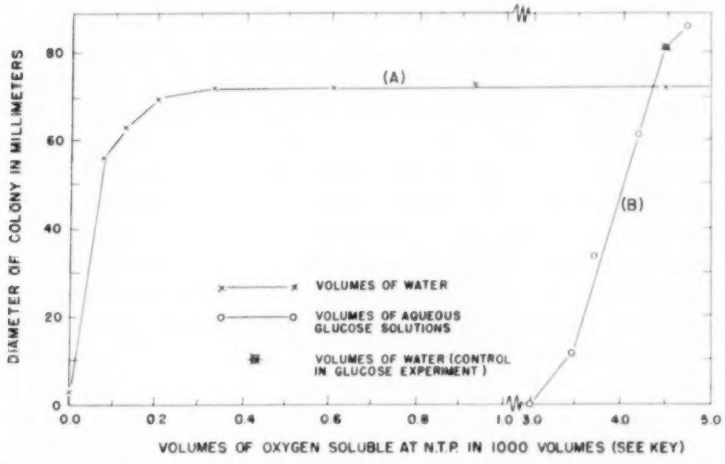

FIG. 1. Growth curves of *G. candidum.* (A) Curve when grown at varying pressures of oxygen; soluble oxygen calculated in terms of solubility in water (Miller and Golding, 1949). (B) Curve when grown at various concentrations of glucose; soluble oxygen calculated in terms of solubility in aqueous glucose solutions except for control. Partial pressure of oxygen was 19.3%.

basal and enriched plated media inoculated with *A. niger* were incubated at 35° C for 3 days, with *A. flavus* at 32.2° C for 4 days, with *G. candidum* at 29.4° C for 7 days, with *P. notatum* at 26.7° C for 7 days, with *P. roqueforti* at 23.9° C for 7 days, and with *P. expansum* at 21.1° C for 7 days. These temperatures are optimum or near the optimum for growth of the particular organisms. Saturated aqueous solutions of $(NH_4)_2SO_4$ maintained the relative humidity of the atmosphere in the incubation chamber at about 80 percent. Specific reagents and equipment used in a particular experiment are listed under the description of the experiment.

EXPERIMENTAL RESULTS AND DISCUSSION

A. Amount of Oxygen in Solution Depressed to a More Favorable Level. In Fig. 1, a growth curve of *G. candidum* from data compiled by Miller and Golding (**1949**) is compared with growth of the same organism from data compiled by the writers. The former investigators cultured the mold at a normal atmospheric pressure but at a greatly reduced partial pressure of oxygen; nitrogen was used as the diluting gas. The writers cultured the mold at the partial pressure of oxygen in air but on malt agar enriched with various amounts of glucose. In both instances the basal medium was malt agar; the period of incubation was 7 days; the temperature of incubation was about 29.5° C; and the relative humidity was approximately 80 percent. All points on both curves are the average of 5 or more replicates. The diameter of the colony is plotted against the volume of oxygen at normal temperature and pressure (N.T.P.) soluble in 1000 volumes of water in the former case and against the volume of oxygen at N.T.P. soluble in 1000 volumes of glucose solution in the latter case. It was assumed that the solubility of oxygen in malt agar and in malt agar concentrated with glucose is approximately the same as the solubility of this gas in equivalent amounts of water and of aqueous glucose solutions, respectively. The volumes of soluble oxygen were calculated by applying the same formula used by Miller and Golding (**1949**):

$$\frac{\text{mm pressure less vapor pressure}}{760} \times \frac{\text{partial pressure of oxygen}}{110} \times \text{oxygen absorption coefficients for water or for glucose solutions (20° C)} \quad \text{International Critical Tables, 1926} \times 1000 = \text{Volumes oxygen at N.T.P. soluble in 1000 volumes of water or of glucose solution}$$

In the case of this organism, a depressed oxygen supply was not the cause of differences in the rate of growth on concentrated substrates. A similar comparison could be made with the other test molds. Miller and Golding (**1949**) reported that they observed no appreciable influence on the growth of the same six cultures used in this study until the volume of oxygen was below 0.8 volume of oxygen at N.T.P. soluble in 1000 volumes of water. Data collected by other investigators indicate similar results with other fungi, although they are not stated in terms of dissolved oxygen. Theoretically, approximately 2.11 volumes of oxygen at N.T.P. are soluble in 1000 volumes of an eight molal aqueous glucose solution. There is sufficient oxygen, yet very few molds are able to grow on or in such a highly concentrated substrate.

B. The Additional Glucose Served as an Added Source of Nutrients. This point was first checked by substituting a substance not containing a source of carbon in place of glucose. Sodium chloride in amounts purported to be isosmotic with the concentrations of glucose (von Mayenburg, **1901**) generally used in this study was used. FIG. 2 pictures the results and reveals an initial stimulation of growth similar to that due to glucose.

A further check was the determination of the maltose-nitrogen ratio of this particular supply of malt agar. If this ratio were low, then the

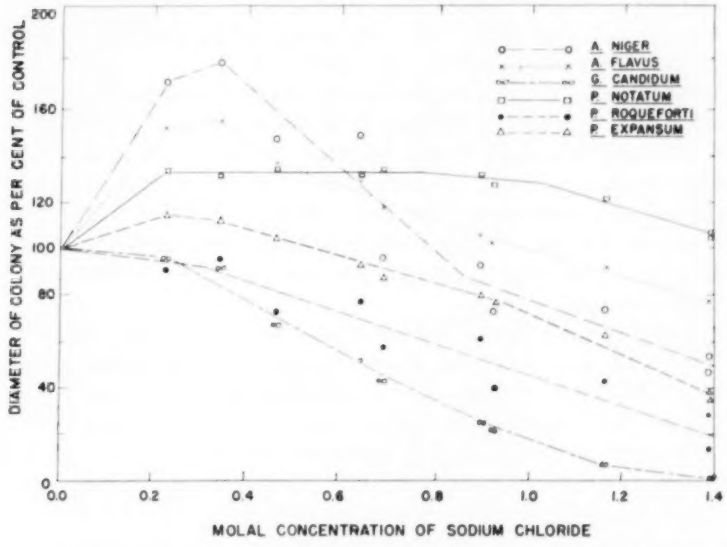

FIG. 2. Effect of various concentrations of sodium chloride on mold growth.

addition of glucose might have accounted for the increase in growth rate. The maltose-nitrogen ratio for malt agar was 118.4 : 1.

Such a large ratio, along with the results of the first experiment, indicated that the additional glucose probably did not serve as a nutrient source. The possibility existed that the hexose was utilized preferentially over the disaccharide by these molds. As all the molds used in this study produced abundant growth on maltose, it was apparent that they possess the enzyme maltase. Elimination of the need of enzymatically splitting the maltose to the more readily utilized hexose could well have resulted in an apparent growth stimulation. That similar stimula-

tion was obtained with sodium chloride, sucrose, and glycerol eliminated this proposal from further consideration.

C. The Oxidation-Reduction Potential of the Medium Was Altered to a More Favorable Level. E_h readings were made on malt agar and on malt agar enriched with graded amounts of glucose (0–100 g per 100 ml of water in increments of 10 g) before and after sterilization in the autoclave. A Beckman pH meter, industrial model, equipped with a platinum electrode was used to measure the potentials. The readings were made at 45° C, so that the agar-containing medium was a sol.

Prior to autoclaving, the E_h varied only 0.012 volt between the lowest and highest readings. The average reading was 0.348 volt. Following autoclaving, the E_h varied 0.065 volt between the lowest and highest readings. The average reading was 0.144 volt.

A change of 0.065 volt in the oxidation-reduction potential of the basal medium by the addition of glucose is of small magnitude and suggests that this factor has slight, if any, influence on the type of growth curve yielded by these molds. Moreover, the data of other investigators strengthen this assumption (Robbins, **1937**; McAlister, **1938**). Buchanan and Fulmer (**1928**) remark that microorganisms apparently show some capacity for self-regulation of the oxidation-reduction level of their substrates.

D. The pH of the Basal Medium Was Changed to a More Favorable Level. Malt agar, malt agar plus various amounts of sodium chloride (1.38 g to 8.25 g per 100 ml water), and malt agar plus various amounts of glucose (10 g to 60 g per 100 ml water) were autoclaved at 119° C for 15 minutes and then tested for hydrogen-ion concentration. A Beckman glass electrode pH meter was used. Only a slight rise in hydrogen-ion concentration occurred with either substance (0.26 pH unit with sodium chloride and 0.34 pH unit with glucose).

These results agree with those of Shaw (**1935**) who found that varied carbohydrate concentrations sterilized with his culture medium did not change the initial pH significantly. Baumgartner (**1937**) observed that the addition of sodium chloride to nutrient medium lowered the pH value 0.2 of a pH unit. When Anderson *et al.* (**1949**) added concentrations of 1, 2, 4, and 8 percent sodium chloride to tomato juice, the initial pH value was lowered from 4.5 to 4.0 as the salt concentration increased. On the other hand 10, 25, 40, and 50 percent solutions of either sucrose or glucose did not alter the pH value of the tomato juice.

That many molds grow rapidly over a rather wide pH range is well known (Thom, **1910**; Talice, **1930**; Wolf and Wolf, **1947**; Hawker, **1950**; Lilly and Barnett, **1951**). It is obvious that slight pH changes

can hardly be a significant factor in influencing the growth curves under consideration.

E. Growth Was Influenced by Osmotic Pressure Changes. Equal quantities of sucrose (U and I), glucose (Cerelose), glycerol (Eastman Kodak), and ethylene glycol (Eastman Kodak) in terms of grams per

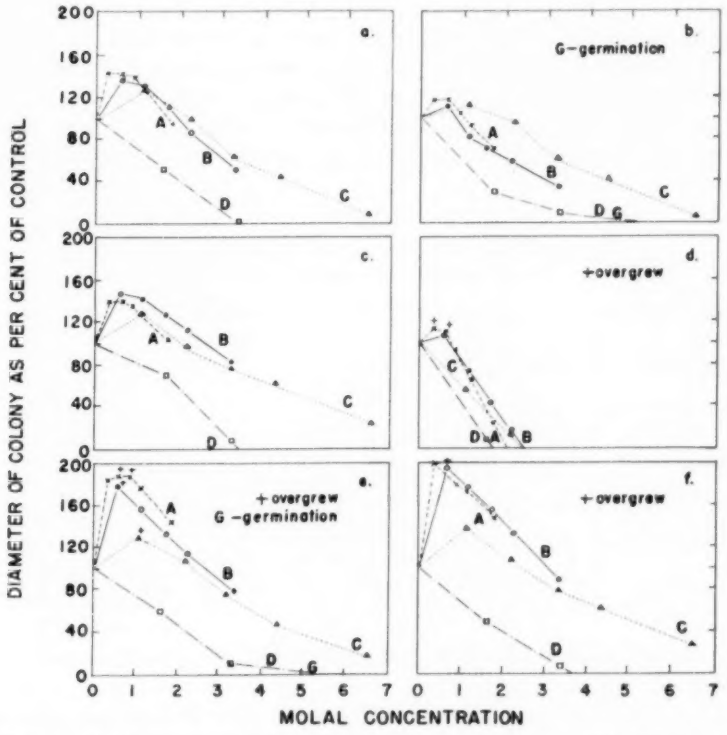

FIG. 3. Molds grown on malt agar containing (A) sucrose, (B) glucose, (C) glycerol, (D) ethylene glycol. a. *P. expansum:* incubation for 7 days at 21.1° C. b. *P. roqueforti:* incubation for 7 days at 23.9° C. c. *P. notatum:* incubation for 7 days at 26.7° C. d. *G. candidum:* incubation for 7 days at 29.5° C. e. *A. flavus:* incubation for 4 days (glycerol 5 days) at 32.2° C. f. *A. niger:* incubation for 3 days (glycerol and ethylene glycol 4 days) at 35° C.

100 ml of water were added to the malt agar. The concentrations have been converted into terms of molality in FIG. 3 to facilitate comparison of the results on the basis of osmotic pressures. Evidences of toxicity, availablity as a carbon source, and osmotic concentration influences were

thus made more obvious. Instead of molal concentrations, calculated osmotic pressures in atmospheres could have been used as the abscissa. However, since the osmotic pressures of solutions containing equal weights of non-electrolytes vary inversely with the molecular weights of the solutes (Philip, **1925**), the same relations between the compounds were manifested when molal concentrations were used. The osmotic pressures in atmospheres exerted by the lowest and highest molal concentrations of the various compounds were calculated (Daniels, **1948**) and are tabulated in TABLE I. Because, as with gases, the concentration-temperature relationship holds only for extremely dilute solutions, these figures must be accepted as approximations with the inherent error increasing directly with the concentration (Philip, **1925**). Despite this,

TABLE I

CALCULATED OSMOTIC PRESSURES IN ATMOSPHERE OF MOLAL CONCENTRATIONS OF VARIOUS COMPOUNDS

Compound	Gram mol. wt.	Molal concentration	Temperatures °C					
			35.0	32.2	29.5	26.7	23.9	21.1
Sucrose	342	0.292	7.39	7.32	7.26	7.20	7.13	7.06
		1.752	44.34	43.92	43.56	43.20	42.78	42.36
Glucose	180	0.555	14.00	13.90	13.70	13.60	13.50	13.40
		3.330	84.00	83.40	82.20	81.60	81.00	80.40
Glycerol	92	1.086	27.40	27.20	26.90	26.70	26.40	26.20
		6.516	164.40	163.20	161.40	160.20	158.40	157.20
Ethylene Glycol	62	1.610	40.70	40.30	39.90	39.60	39.20	38.80
		9.660	244.20	241.80	239.40	237.60	235.20	232.80
Malt Agar	371.6	0.012	3.06	3.03	3.00	2.98	2.95	2.92

the slopes of the curves, in most instances, indicate that the error may not be so large as supposed.

Also listed in TABLE I are the calculated osmotic pressures exerted by 4.5 g of malt agar per 100 ml of water at the 6 different temperatures of incubation. These values were obtained by first determining the freezing point of malt agar dissolved in cold, distilled water and then, by means of this value, determining the gram molecular weight of malt agar (Daniels, **1948**). The effect of the complex, high-molecular-weight carbohydrate, agar-agar, was considered negligible since the calculated gram molecular weight for malt agar was 371.6 g. This is but slightly more than the gram molecular weight of maltose (342), indicating that the osmotic influence of the malt agar is probably dependent chiefly on the presence of the disaccharide.

A second study compared the effect of isosmotic concentrations of glucose and sodium chloride (von Mayenburg, **1901**) upon growth. These results are presented in FIGS. 4 and 5.

The first study, comparing the effect of molal concentrations of glucose, sucrose, glycerol, and ethylene glycol on growth (FIG. 3), definitely

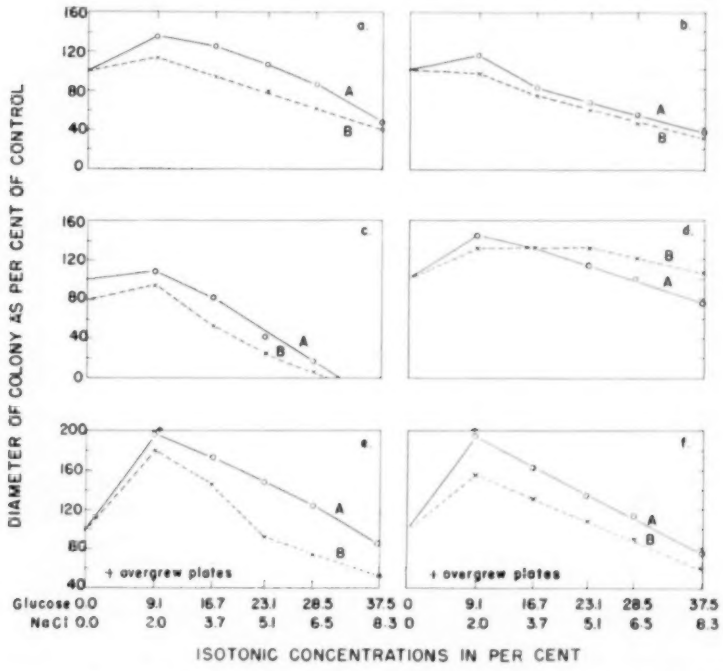

FIG. 4. Molds cultured on malt agar concentrated with isotonic concentrations of (A) glucose and (B) sodium chloride. a. *P. expansum:* incubation for 7 days at 21.1° C. b. *P. roqueforti:* incubation for 7 days at 23.9° C. c. *G. candidum:* incubation for 7 days at 29.5° C. d. *P. notatum:* incubation for 7 days at 26.7° C. e. *A. niger:* incubation for 3 days at 35° C. f. *A. flavus:* incubation for 4 days at 32.2° C.

illustrated the toxicity of ethylene glycol for all the test organisms. Although good osmotic pressure correlation between the different compounds was noted, only in the case of *P. expansum* was this correlation observed for the three compounds. Glycerol was not as stimulatory a carbon source for the Aspergilli as glucose or sucrose. Steinberg (**1942a, b**) noted the absence of vigorous growth by his strain of *A.*

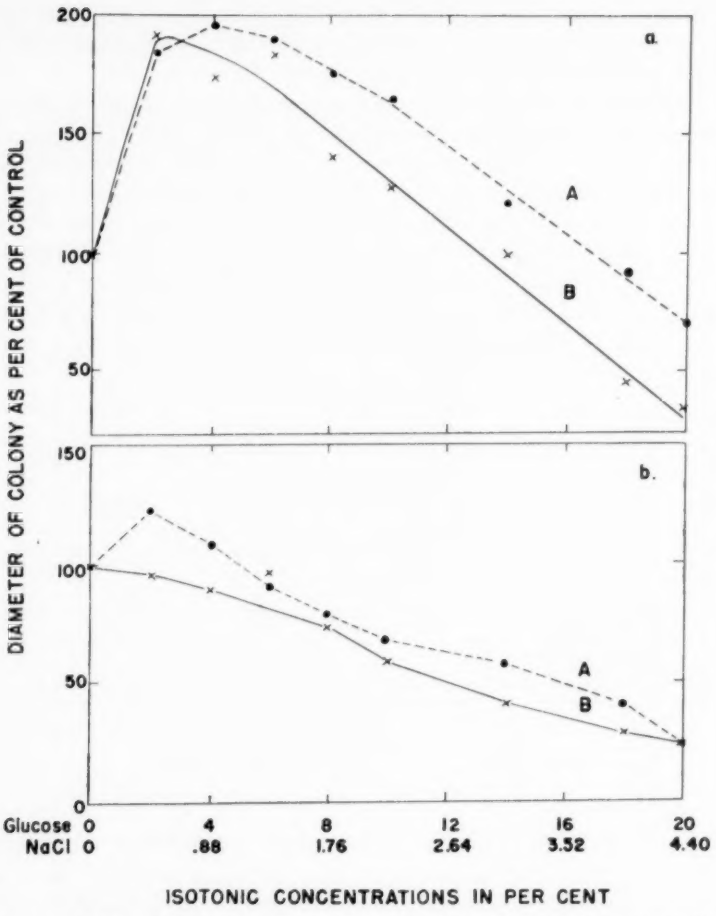

FIG. 5. Molds cultured on malt agar concentrated with isotonic concentrations of (A) glucose and (B) sodium chloride. a. *A. niger:* incubated 67 hours at 37° C. b. *P. roqueforti:* incubated 7 days at 23.2° C.

niger when glycerol was used as the source of carbon. At higher concentrations, this compound was tolerated better than either glucose or sucrose by *P. roqueforti,* despite the higher osmotic pressure of these concentrations of glycerol.

Results of the second study, comparing the effect of isotonic concentrations of glucose and sodium chloride upon the molds (FIGS. 4 and 5), also emphasized the differences in response of these organisms to envi-

ronmental factors. The two organisms, *G. candidum* and *P. roqueforti*, which yielded the smallest growth stimulation with added glucose, showed no stimulation with added sodium chloride; instead, they showed slightly decreased growth at the lowest concentration (0.44 percent). Increased concentrations of sodium chloride were obviously toxic to both *A. niger* and *P. expansum*. Osmotic pressure correlation was best for *P. roqueforti*. As the concentrations of glucose and sodium chloride were increased, growth of *P. notatum* remained at a higher level and decreased more gradually with sodium chloride than with glucose.

An obvious but varying degree of osmotic pressure correlation exists in these growth curves. The results of these studies indicate that osmotic influences are an important, although not the sole, factor in the characteristic growth of these molds on progressively concentrated substrates.

GENERAL DISCUSSION

The resistance of molds to high osmotic pressures and, consequently, their growth on highly concentrated substrates has often been reported. Several investigators have demonstrated that the osmotic pressure of fungus plant pathogens is considerably greater than that of their hosts (Hawkins, **1916**; Thatcher, **1939**). How fungi accomplish this has been the subject of investigations for a number of years.

Eschenhagen in 1889 (von Mayenburg, **1901**; Hawkins, **1916**) postulated that fungi are able to grow in highly concentrated solutions because they produce osmotically-active substances within their cells. Although von Mayenburg (**1901**) was unable to isolate such a substance, he concluded that it is an easily decomposed, carbon-containing compound of about the same molecular weight as glycerin.

The present study not only re-emphasizes the resistance to high osmotic concentrations of some common molds but also indicates the existence of an optimum osmotic concentration of the substrate. Buchanan and Fulmer (**1928**) concluded that microorganisms in general frequently grow best in solutions having a definite osmotic concentration, and that compounds bringing about this optimum osmotic pressure may act as indirect growth stimulants. Galloway (**1935**) observed such a stimulation of growth in some molds responsible for mildew of textiles but did not attempt an explanation.

The importance of osmotic pressures as one of the environmental factors determining the predominance of certain molds on particular substrates was emphasized by the sensitivity of *G. candidum* and *P. roqueforti* to high osmotic concentrations. The former organism pre-

dominates in the spoilage of milk and the latter is important in the ripening of certain cheeses. These substrates are relatively inert osmotically, milk having a remarkably constant osmotic pressure of 6.78 atmospheres (Davis, **1950**). Published data (Thom *et al*, **1914**; Hammer, **1948**) suggest that the predominance of *P. roqueforti* in blue cheese can be attributed to this mold's tolerance of low oxygen tensions, high carbon dioxide tensions, and high salt concentrations better than other molds. Thom (**1910**) has shown that *G. candidum* is also tolerant of high carbon dioxide and low oxygen tensions. Golding's study of factors affecting the growth of four strains of *P. roqueforti* (**1926**) revealed that a general statement as to the salt tolerance of *P. roqueforti* cannot be made. On the basis of the experimental results described earlier, it is better to attribute the predominance of *P. roqueforti* in blue cheese and of *G. candidum* in milk, cream, and cheeses, under conditions of temperature and humidity at which these products are usually held, to (1) their tolerance of high carbon dioxide tensions and (2) their faster rate of growth than other molds on substrates of low osmotic concentration.

The toxicity of ethylene glycol for all the test organisms is in accord with the report of Neidig (**1913**) who used this substance as the sole carbon source in a modified Czapek's medium. None of his cultures produced normal colonies in the course of 3 weeks' incubation. Strains of *A. niger, P. expansum,* and *P. roqueforti* were among his test organisms.

That there is no simple explanation for this effect on fungus growth has been shown by the investigation of four other possible causes of this phenomenon. Moreover, this list of tentative causes was limited arbitrarily. For instance, Foster (**1939**) states that, ". . . the need for trace amounts of various heavy metals such as zinc, iron, manganese, copper, and possibly others is a phenomenon widespread among the fungi." The addition to the medium of large amounts of sugar or other substances, unless these substances have been rigorously purified, could well include enough trace elements to raise the level of one or another deficient element to the point where accelerated growth would occur.

The sterilization of concentrating substances with the basal medium could alter this medium so that stimulation or retardation of growth would occur. Fulmer *et al* (**1927**, **1931**) reported such stimulation of growth for a strain of yeast and several strains of bacteria when glucose or sucrose and salt solutions were mixed and autoclaved. The results of an experiment in which malt agar and glucose were sterilized separately indicated that this is not the case with these molds.

Possibly the concentrating substances alter the permeability of the

cells as suggested by Ciferri (**1941**) in connection with the dissociation of yeasts. Other investigators have proposed that observed altered physiological responses of various microorganisms to certain substances may be due to their effect on the permeability of the cell membrane (Steinberg, **1919**; Fay, **1934**; Barker, **1936**). No experimental work was done by the writers to correlate growth stimulation with changes in cellular permeability.

The better growth of *P. roqueforti* at high concentrations of glycerol than at high concentrations of either glucose or sucrose may be due to the more rapid metabolism of this compound. Greater permeability to glycerol as well as the highly active, water-soluble lipase produced by *P. roqueforti* (Hammer, **1948**) may also be of importance in this respect.

Another result worthy of note was the far greater tolerance of *P. notatum* to high salt concentrations than to isotonic concentrations of glucose. That sodium chloride altered cell permeability favorably for continued growth is only supposition. Nevertheless, it is difficult to judge how else sodium chloride could have acted to affect growth.

Hawkins (**1913**) concluded that the chemical characteristics of salts and other substances and the physico-chemical properties of their solutions must be thoroughly investigated before a suitable explanation of the relation of osmotic concentrations to fungus growth can be obtained. It is certain that no simple, general explanation of the relation of the varied responses to osmotic concentrations between molds and even between strains of a species (Golding, **1926**) is possible.

SUMMARY

Spores of *Aspergillus niger*, *A. flavus*, *Geotrichum candidum*, *Penicillium notatum*, *P. roqueforti*, and *P. expansum* were cultured on malt agar variously concentrated with sucrose, glucose, glycerol, ethylene glycol, or sodium chloride. The following causes for the characteristic growth of these fungi on such concentrated substrates were tentatively proposed and subsequently investigated: (1) oxygen in solution was depressed to a level more favorable for growth; (2) the additional glucose served as an added source of nutrients; (3) the oxidation-reduction potential of the medium was altered to a more favorable level; (4) the reaction of the basal medium was changed to a more favorable level; and (5) growth was influenced by osmotic concentration changes. Other possible causes of stimulated growth considered are: (1) increased quantities of deficient trace elements as impurities in the concentrating substances, (2) changes in the growth-promoting potential of malt agar

by sterilization with the concentrating substances, and (3) effect of the concentrating substances on cell permeability.

The results indicate that osmotic pressure changes are an important factor in shaping these growth curves. It is noteworthy that:

1. Ethylene glycol was toxic for these organisms under the conditions of this study.
2. *G. candidum* and *P. roqueforti,* common molds of cheeses and other milk products, were the most sensitive of the organisms to high osmotic concentrations, the former organism showing the largest retardation of growth.
3. *P. roqueforti* displayed an unexpected tolerance to high concentrations of glycerol.
4. Glycerol was not as stimulatory a carbon source for the Aspergilli as were glucose and sucrose.
5. *P. notatum* was more tolerant than the other test organisms to increased sodium chloride concentrations; growth of this organism decreased more rapidly on the glucose-enriched substrate than on the salt-containing medium.

It is concluded that no single explanation can account completely for the growth stimulation resulting when certain substances are added to an adequate medium. Rather, for each case, thorough studies of the chemical characteristics of the substances involved, of the physicochemical properties of their solutions, and of the physiological and morphological responses of the particular organism to these are necessary.

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THE FUNGISTATIC BARRIER EFFECT OF "S-COATED" COTTON USED AS VIAL PLUGS [1]

SARA BACHE-WIIG

The first series of experiments [2] here recorded was undertaken in order to work out a technique of maintaining cultures of dissected *Datura* embryos free from fungus contamination during humid summer weather.[3]

The chemicals tested were two "S-coatings" developed by Dr. Alexander Goetz and his collaborators at the California Institute of Technology. The writer is indebted to Dr. Goetz for kindly providing the S-coated cotton and for the following description of the material and its physical-chemical properties.

"S-coating consists, prior to a surface application, of a paint-like fluid in which an intimate mixture of *two* types of colloidal particles are suspended. One type consists of non-absorptive colloidal carbon (particle size 90 mμ), in which each particle carries traces of silver in a carefully controlled chemi-sorbed state. This type of particle acts as oxidation promoting agent. The other particle type consists of a scarcely soluble inorganic peroxide which acts as oxygen donor. The mixture is suspended in a plastic vehicle which forms after drying micellae which hold the pigment particles in the surface layer but also permit the establishment of 'wetting bridges' between the particles. In this way a surface configuration is produced which has been shown to be, though insoluble, germicidally active for very long periods of time in the sense that it will inactivate microorganisms which come in contact with the surface."

More detailed information on the nature and properties of S-coatings, together with experimental evidence on the efficacy and permanence of the germicidal barriers which these coatings constitute, will be found in articles by A. Goetz, R. Tracy, and S. Goetz (**1942**) and by A. Goetz (**1944**).

[1] Contributions from the Department of Botany, Smith College, No. 55.

[2] Planned with the cooperation of Dr. Mary E. Sanders who also furnished some of the *Datura* embryos. The use of "S-coatings" was suggested by Dr. P. R. Gast.

[3] The author is indebted to Dr. A. F. Blakeslee for suggesting the problem and for criticism. Another series of experiments in which chemicals were introduced into culture media of fungi and of embryos in search of a chemical which might inhibit growth of fungi without inhibiting growth of embryos was abandoned because all chemicals tested proved toxic to embryos.

TABLE I
Series A

Number of vials		Number of vials contaminated at the end of the following number of days:								
		11	25	29	31	37	50	68	99	134
3	Vials plugged with S-coated cotton (formula No. 308–2).	0	0	0	0	0	0	0	0	3
3	Controls. Vials plugged with untreated cotton.	0	0	1	1	3				

Datura embryos were grown in glass vials (70 × 20 mm) in nutrient agar. Vials were plugged before sterilization with untreated cotton. In the control vials these plugs were retained. In the remaining vials they were replaced by plugs of S-coated cotton. These plugs were rather awkwardly formed, using flamed forceps, in an attempt to approach aseptic conditions in handling the S-coated cotton. All cultures were kept in moist, closed, fungus-contaminated glass dishes in order to provide conditions favorable for fungus invasion of the cultures. They were inspected from time to time for indications of visible fungus growth inside the vials. Series B differs from Series A only in the initial inoculation of the top of each plug with spores of *Aspergillus* sp.

Results are given in TABLES I and II.

The tables show that in both series all controls became contaminated before any vial stoppered with S-coated cotton showed fungus invasion.

As for young *Datura* plants, they did not appear to be injured by the

TABLE II
Series B
TOP OF EACH PLUG INOCULATED WITH ASPERGILLUS SP.

Number of vials		Number of vials contaminated at the end of the following number of days:							
		8	12	14	20	33	51	81	112
7	Vials plugged with S-coated cotton (formula No. 308–2).	0	0	0	0	0	3	6	7
*6	Vials plugged with S-coated cotton (formula No. 308–2 C P).	0	0	0	0	0	3	5	6
7	Controls. Vials plugged with untreated cotton.	0	2	2	6	7			

* Reduced from 7 by falling out of plug from one vial.

S-coated cotton even when a growing leaf pressed against a plug of this material.

The experiments reported upon above were carried out in 1946.

Further tests were carried out in 1952. A fresh supply of S-coated cotton was kindly furnished by the A. G. Chemical Co., John J. Vrolyk, Mgr., of Pasadena, Cal. In addition, some treated cotton remaining from 1946 was used in order to test the effect of age on the efficacy of the S-coatings.

In the 1952 series no attempt was made to handle the S-coated cotton aseptically. The plugs were rolled plugs, formed by hand. Just before being substituted for the untreated plugs of the autoclaved vials they were wetted in sterile distilled water, then squeezed to get rid of excess water. This procedure was followed on the advice of Mr. Vrolyk who explained that: "In the dry state, at room temperature, S-coated material is bacteristatic and fungistatic, but as soon as it becomes wet it is for a time highly bactericidal and should sterilize itself within a minute or two." [4]

In Series C, TABLE III, the vials contained nutrient agar (0.8% agar) planted with *Datura* embryos.[5] In Series D, TABLE IV, the vials contained only potato dextrose agar (2.5% agar). The latter were stored for a week before the experiment was started and the medium had dried a little. The vials were kept, as before, in moist, closed glass dishes. They were examined at 7-day intervals for 154 days.

TABLES III and IV show that fungus contamination of vials plugged with cotton recently impregnated with S-coatings was first discerned 42 or more days after all controls had become contaminated, and that some vials plugged with these S-coatings appeared uncontaminated even after 154 days.

The same tables show that when S-coatings were 6 years old contamination was later in vials plugged with this material than in control vials but earlier than when the S-coatings had been recently made.

When corresponding parts of TABLES III and IV are compared, delayed contamination of Series D as compared with Series C may be noted. This can probably be attributed to greater dryness of the medium.

GENERAL CONCLUSIONS AND DISCUSSION

All four series of experiments were consistent in showing no fungus invasion of vials plugged with recently made S-coated cotton until after all control vials had become contaminated. Some vials plugged with the

[4] Letter from Mr. J. J. Vrolyk.

[5] The planted vials were kindly provided by Dr. Sophie Satina.

TABLE III
Series C

Number of vials		Number of vials contaminated at the end of the following number of days:																					
		1	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147	154
*11	Vials plugged with S-coated cotton (No. S-100, repetition of formula 308–2 C P). Cotton heavily impregnated.	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	5	7	7	7	8	8	8
8	Vials plugged with S-coated cotton (No. S-100, repetition of formula 308–2 C P). Cotton lightly impregnated.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	3	3	4
8	Vials plugged with S-coated cotton (308–2 C P) stored for 6 years.	0	0	0	0	0	0	0	0	1	1	1	2	3	3	3	6	8					
12	Controls. Vials plugged with untreated cotton.	0	0	9	10	11	11	12															

* Reduced from 12 by contamination of one vial in initial manipulation.

TABLE IV
Series D

Number of vials		Number of vials contaminated at the end of the following number of days:																					
		7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147	154
8	Vials plugged with S-coated cotton (No. S-100, repetition of formula 308–2 C P). Cotton lightly impregnated.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
8	Vials plugged with S-coated cotton (308–2) stored for 6 years.	0	0	0	0	0	0	0	1	1	1	1	2	3	3	3	4	4	4	4	4	6	7
8	Controls. Vials plugged with untreated cotton.	0	0	1	2	3	4	6	7	8													

treated cotton showed no contamination at the end of the experiment.

Even 6-year-old S-coated cotton was effective in delaying fungus invasion. However, the old S-coatings were less effective than those recently made.

It should also be emphasized that, in most of the control vials, invading fungi were from the first observed sporulating on the inner end of the plug, while in the vials with S-coated plugs fungus growth was in most cases first detected on the inside of the glass tube, indicating that fungi grew through the untreated cotton plug, but that when the plug was of S-coated cotton fungus entry was effected through channels along the side of the vial, not through the coated cotton.

It appeared to make no difference whether the cotton was lightly or heavily impregnated with S-coating, and whether or not the S-coating was of a type (indicated by the letters —C P) more germicidal under acid conditions. (It may be added parenthetically that it also made no difference whether the cotton used was absorbent or non-absorbent.)

The extended protection given by the S-coated cotton plugs under conditions extraordinarily favorable for fungus invasion suggests that they might be tried in cases requiring long maintenance of cotton-plugged cultures of Embryophyta under laboratory or greenhouse conditions without the use of a constant temperature chamber.

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ANTIMYCOTIC EFFECTS OF AN EXTRACT OF CATALPA [1]

ROBERT J. MCGRAY [2] AND E. S. MCDONOUGH [3]

(WITH 3 FIGURES)

The work of Hawley, Fleck and Richards (**1**), and more recently numerous others, has indicated that extractives from wood have fungistatic activities. In several cases these extractives have been tested against wood-rotting fungi, and it has generally been considered that at least some of the durability of certain woods is caused by extractable substances. Such substances have also been shown to inhibit the growth of other fungi, including human pathogens (**3**). *Catalpa speciosa* Warder has yielded a water soluble material (**1**) and an alcoholic extractive (**2**) which have been shown to inhibit the growth of some wood-decaying fungi. Extracts from catalpa stored in alcohol have been shown to retain their antiobiotic capacities indefinitely, but water extracts have been found to lose this property rapidly. It has also been reported that there is a negative correlation between fungistatic capacity of extractives and the length of time catalpa wood is stored (**2**). This report presents the results of an investigation designed to survey the growth-inhibiting powers of the alcoholic extract against some saprophytes, plant parasites, and human pathogenic fungi.[4]

MATERIALS AND METHODS

The extractive was prepared as follows: 100 gms of shavings of fresh-cut catalpa heartwood were ground in a blender and soaked in

[1] A portion of this work was incorporated in a thesis submitted to the Graduate School of Marquette University in partial fulfillment of the requirements for the degree of Master of Science.

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[3] Biology Department, Marquette University, Milwaukee 3, Wisconsin.

[4] The authors thank Dr. Michael Furcolow, U. S. Public Health Service, University of Kansas Medical Center, for furnishing cultures of *Histoplasma capsulatum;* Plant Pathology Department of the University of Minnesota for cultures of wood-destroying fungi; and Dr. Libero Ajello, Laboratory Division, U. S. Public Health Service, Atlanta, Georgia, for all the other cultures of human pathogens.

one liter of 95% ethanol for two weeks. The mash was then filtered and the solvent completely evaporated from the filtrate, leaving a brown residue which weighed 4.1 gms. This was redissolved in 95% ethanol, making a solution having in 1 ml the extractive from one gm of wood. This solution, which had a pH of approximately 5.5, showed 43% transmittance at 660 mμ, using 7/8″ absorption cells in an Evelyn photoelectric colorimeter set at 100% transmittance with an ethanol blank.

Pieces of Whatman No. 2 filter paper 1″ by ½″ were soaked for 15 minutes in the solution and dried at 160° C for two hours. Papers soaked in ethanol only and sterilized were used in control plates. Two pieces of dried extract-impregnated paper were placed 1″ apart on the surface of a Sabouraud dextrose agar plate (dextrose 4%, peptdone 1%, agar 1.5%, pH 5.5). A ¼″ square block containing young mycelium was cut out of a 2–5-day-old culture plate of the fungus to be tested and transferred to the center of the agar plate between the two pieces of paper. Both experimental and control plates in all tests were prepared in duplicate and were incubated at 30–32° C in constant humidity. The plates were removed from the incubator each day for growth measurements. Readings were continued up to the time when radial growth ceased or until the plates were covered with mycelium.

Radial growth was measured in two directions: in a line perpendicular to the long axis of the filter paper (cross growth), as well as parallel to it (parallel growth). The percent of relative growth was calculated by dividing the amount of cross growth in the experimental plate less ¼″ (the width of the agar containing mycelium used for inoculation), with the corresponding figure for the control. This figure subtracted from one and multiplied by 100 gave the percent retardation. Since few fungi grow in culture at the same rate, it was impossible to set an arbitrary date during the incubation period at which time comparable measurements could be taken. For this reason the day on which the greatest difference in growth occurred between the experimentals and the controls was used to determine figures of percent inhibition for purposes of comparison.

EXPERIMENTAL RESULTS

Inhibition of radial growth was observed for all fungi tested. Duplicate plates showed no significant differences in growth characteristics, and the average of the two cross-growth figures was used to calculate the percent inhibition. Differences in the amount of inhibition, length of time at which the maximum amount of inhibition took place and changes in cultural characteristics were the most apparent variations

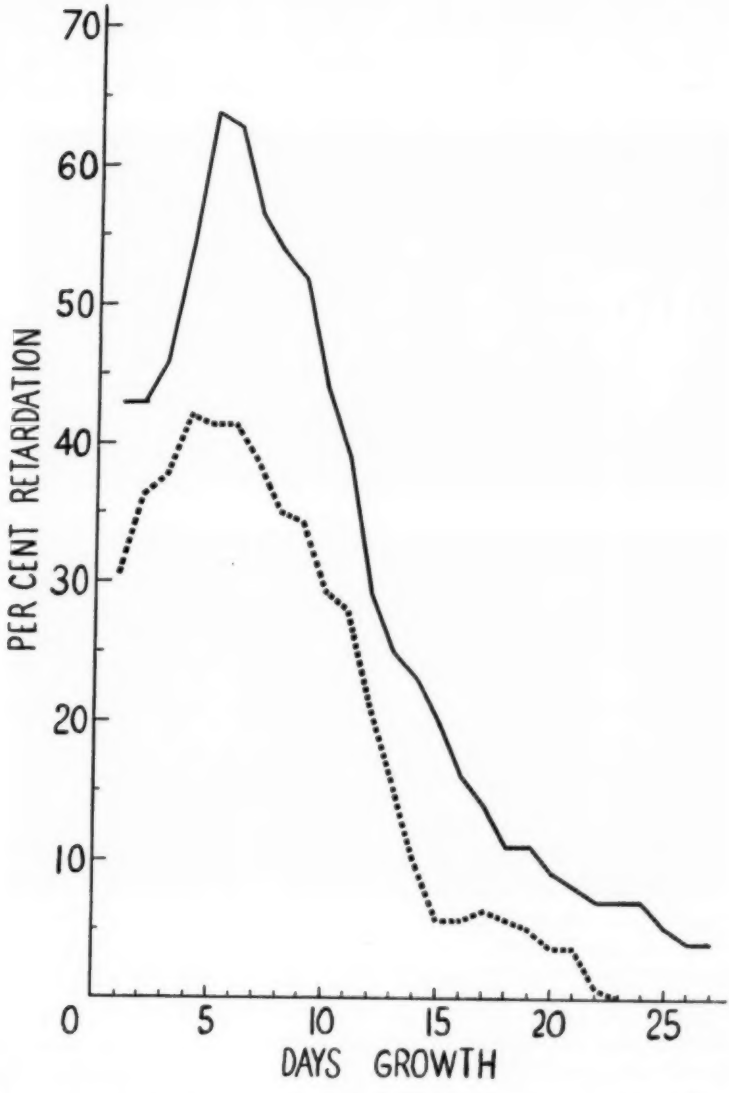
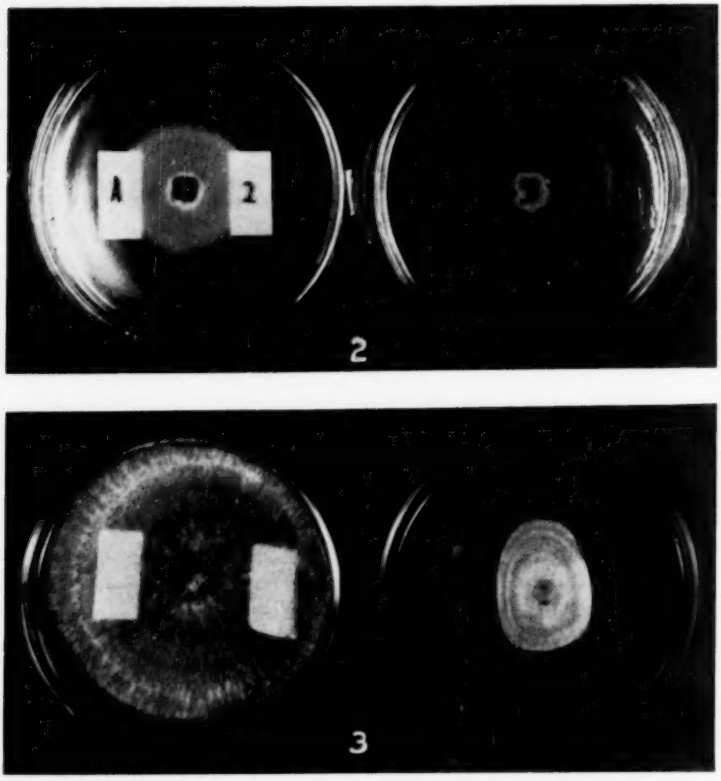


Fig. 1. Inhibition of *Cephalosporium*. Based on daily percent retardation of cross growth (solid line) and parallel growth (dotted line).

noticed throughout the survey. A curve based on the daily comparisons of the growth in experimentals and controls characterized each fungus, the high point of the curve corresponding to the maximum inhibition of the test organism as shown in FIG. 1 for *Cephalosporium*. Parallel



FIGS. 2, 3. 2. Seventeen-day cultures of *Histoplasma capsulatum* × 970. A2. Control. C2. Experimental. 3. *Rhizoctonia solani*. Experimental on the right. This photograph was made from a preliminary run and measurements for TABLE I were not made from these plates.

growth curves resembled the cross-growth curves, but, as might be expected, the retardation of growth was less in parallel growth. The shape of the curve was no doubt influenced by the growth rate of the fungus, the rate of diffusion of the extractive, the sensitivity of the fungus and the rate of breakdown of the fungistatic material. These factors likewise influenced the shape of the mycelial mat, this fluctuating

in time. Very sensitive organisms, such as *Histoplasma capsulatum* (FIG. 2), tended to show a square or circular growth pattern while more rapidly growing or less sensitive organisms, such as *Rhizoctonia solani*, showed a more ellipsoidal pattern (FIG. 3).

TABLE I

INHIBITION OF VARIOUS FUNGI USING THE DUPLICATE IMPREGNATED FILTER-PAPER METHOD

FUNGUS	MAXIMUM INHIBITION		TOTAL GROWTH PERIOD
	Percent	Day	
Allescheria boydii Shear	34	8	25
Alternaria sp.	46	5	13
Aspergillus niger v. Tiegh	31	3	5
Cephalosporium sp.	65	5	26
Epidermophyton floccosum (Harz) Langeron and Milochevitch	56	3	30
Fusarium sp.	65	4	15
Helminthosporium sp.	56	5	25
Histoplasma capsulatum Darling (Chamblee strain)	93	3	30
H. capsulatum Darling (Kansas strains)			
970	99	9	26
531	95	5	22
2132	94	5	19
A136	96	7	26
2012	95	5	26
2451	95	5	26
2464	94	5	18
2474	90	5	26
Microsporum audouini Gruby	61	5	24
M. gypseum (Bodin) Guiart and Grigorakis	57	7	23
Paecilomyces sp.	51	4	6
Phialophora verrucosa Thaxter	67	4	27
Polyporus sulfureus Fr.	64	2	26
P. versicolor Fr.	78	1	10
Poria microspora Overh.	85	3	30
Pythium debaryanum Hesse	64	2.5	5
Rhizoctonia solani Kuhn	72	16	26
Rhizopus nigricans Ehrenberg	40	2	3
Schizophyllum commune Fr.	43	5	20
Syncephalastrum sp.	36	2	3
Trichoderma sp.	41	1	5
Trichophyton rubrum (A. Cast.) Sabouraud	58	5	30

Inhibition of pigment formation was noticed during the testing of several species, especially *Rhizoctonia* and *Helminthosporium*. In many instances structural characteristics of the mycelium were also altered. Experimental plates of *Rhizoctonia solani* took on a compact, thickly cottony appearance which was very light in color as opposed to the growth in control plates, where sparsely threaded, flat and tan mycelium resulted (FIG. 3).

In TABLE I are listed alphabetically the fungi tested by the duplicate impregnated-filter-paper method. These fungi may also be grouped as human pathogens, plant pathogens and common molds. Of the undoubted human pathogenic fungi *Microsporium audouini, M. gypseum, Trichophyton rubrum* and *Epidermophyton floccosum* are classified as dermatophytes. These were retarded in the order of most to least inhibition as listed here. *Allescheria boydii* had a low percent inhibition but *Phialophora verrucosa,* another subcutaneous pathogen, ranked near the mean in this respect. Of all fungi tested, *Histoplasma capsulatum,* which causes a deep mycosis in man, had the maximum percent inhibition. As noticeable as its inhibition was the change in cultural characteristics of this fungus. In general, the control was loosely cottony in appearance, spreading in a low, flat growth; the colonies in the experimental plates were densely cottony and the mycelium was raised into a high tuft immediately surrounding and covering the original inoculum. Of the wood-rotting organisms studied *Poria microspora* was inhibited most (85%), while *Schizophyllum commune* was inhibited least (43%). As a group the wood-rotting fungi had the highest percent inhibition, the human pathogenic fungi were slightly less inhibited, and the saprophytic fungi were least retarded by the extractive, *Aspergillus niger* having the lowest percent (31) of all fungi studied.

DISCUSSION

The results of this investigation showed that the extractive retarded the growth of fungi in general, including members of all the great taxonomic groups of mycelioid forms. Much variability existed in the sensitivity of fungi to the antimycotic and, while the limited number of species studied (22) was not large enough to allow generalizations with certainty of accuracy, it was interesting that wood-rotting fungi were most inhibited, human pathogens ranked second and saprophytes were least retarded. On the other hand, each of these groups was found to contain relatively resistant (*Aspergillus niger,' Allescheria boydii* and *Schizophyllum commune*) and relatively sensitive (*Cephalosporium, Poria microspora* and *Histoplasma capsulatum*) forms, thus making it impossible to classify fungi in regard to pathogenicity on their reactions to the extractive from catalpa alone.

Polyporus versicolor Fr. has been reported to cause a spongy yellow heart rot and *Schizophyllum commune* Fr. a sapwood rot of catalpa (4). Since the growth of these fungi has been shown to be retarded by extractives from catalpa, a study on the ability of these wood rotters to cause

primary infection as correlated with the fungistatic materials in the tree would seem to be indicated.

Further study on the purification of catalpa extractives and an investigation of the effects of the purified material, or materials, on microorganisms and on animals seem justified by the results obtained.

The authors consider that the method of testing inhibition used in the experiments is rapid and sufficiently accurate for an original assay of fungistasis and for purposes of comparison. While the actual figures of percent inhibition would no doubt vary with repetition, in practice these variations were not large. *Schizophyllum commune* was used throughout the survey as a control on the effectiveness of the fungistatic material. The percent inhibition obtained in all tests coincided closely.

SUMMARY

A water labile and relatively heat and ethanol stabile substance obtained from the heartwood of *Catalpa speciosa* Warder was assayed as to its fungistatic activity by a duplicate impregnated-filter-paper method. Twenty-two species representing all the higher categories of mycelioid fungi were studied. All organisms studied were more or less retarded. As a group the wood-rotting fungi had the highest percent inhibition, the human pathogenic were slightly less inhibited and the saprophytes were least retarded.

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NUCLEAR PHENOMENA IN THE BASIDIA AND BASIDIOSPORES OF OMPHALIA FLAVIDA [1]

LUIS SEQUEIRA [2]

(WITH 20 FIGURES)

The most important disease of coffee in Latin America is without any doubt that caused by *Omphalia flavida* Maubl. & Rangel and commonly known by various vernacular names such as: American leaf-spot disease of coffee, "ojo de gallo," "viruela," etc. In countries such as Costa Rica, Guatemala, and Colombia, this disease has been estimated to cause an annual loss of several million dollars.

Characteristically, on the leaves of coffee and many other host plants, this organism causes round or ovoid spots, 0.4 to 1 cm in diameter, followed by severe premature defoliation. If fallen leaves bearing these fungous lesions remain under conditions of extremely high constant humidity there appear, usually at the edge of the spots, a small number of yellow structures which push their way through the epidermis and develop into small but characteristic basidiomycete sporophores, which represent the perfect stage of *Omphalia flavida*. Under natural conditions these are very seldom found, but Ashby (1) and Buller (2) were able to obtain this perfect stage on standard culture media and have given us detailed accounts of the distinctive structural characteristics of these sporophores.

The perfect stage of *Omphalia flavida* is of considerable scientific interest not only because of its extreme rarity in nature, but also because it represents a fundamental link in our knowledge of the life history of this fungus. In the course of extensive investigations of various aspects of the development, reproduction, and parasitism of this destructive fungus the writer has made a cytological study of the nuclear behavior in its perfect stage. It is the purpose of this paper to present certain unusual and interesting features of the nuclear cycles revealed.

[1] This paper is a portion of a thesis submitted to the Faculty of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology, May 1952.

[2] Parker Fellow of Harvard University, 1952–1953.

MATERIALS AND METHODS

Cytological investigations of *Omphalia flavida* are hindered because the sporophores produced on standard culture media (FIG. 1), like those collected in nature, are usually abortive and it is only in rare cases that the basidia reach maturity. Even when the fungus is inoculated on live material, such as leaves of coffee or *Bryophyllum calycinum*, the agaric sporophores are produced in small numbers and are generally abortive.

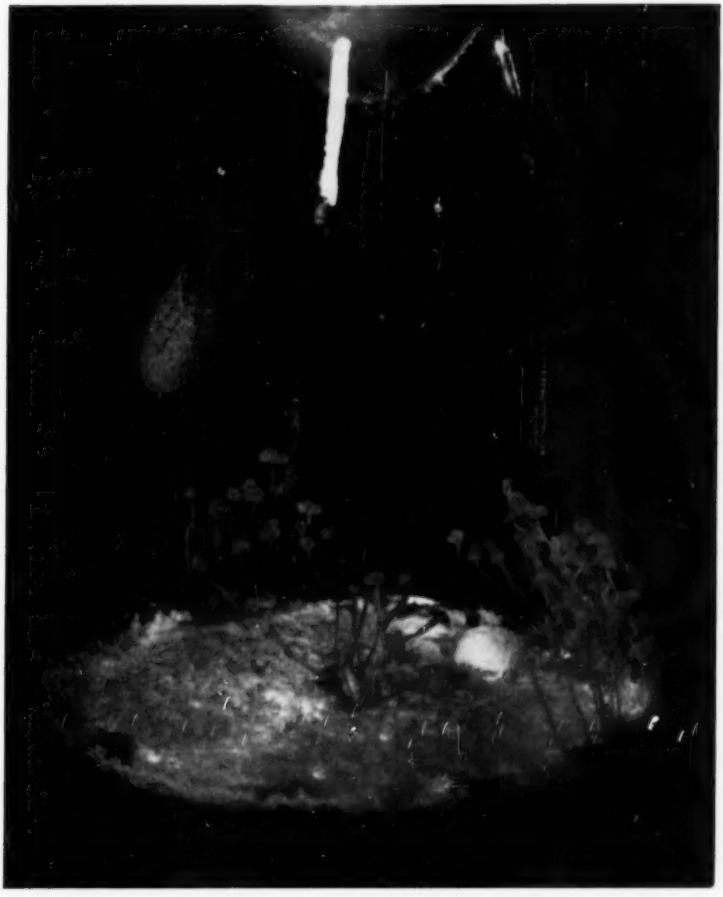


FIG. 1. Typical potato-dextrose agar culture of *Omphalia flavida*. Note the characteristic clumps of long, irregular sporophores. Culture 4 weeks old.

A large number of crude culture media were tested for sporophore production and the most satisfactory results were obtained using millet or unhusked rice seeds. Media made from these two sources were used almost exclusively in this work and on them was grown strain T_w, which in a series of comparative tests of the twelve strains studied had been found to produce the greatest number of normal sporophores with functional basidia.

For general purposes, 10 grams of seeds were placed in a 125 cc Erlenmeyer flask, 30 cc of tap water were added, and the medium was autoclaved for 20 minutes at 15 lbs pressure. After autoclaving, the medium is in the form of a loose granular paste on which the fungus grows very rapidly, each culture producing within 3 to 4 weeks up to 50 sporophores; of these, a reasonable number, about 10%, can be expected to reach maturity and produce basidia.

The standard fixing and sectioning methods commonly used by all other workers in the Hymenomycetes proved ineffectual, because of the very delicate nature of the lamellae and the very poor differentiation of the nuclei. However, the more modern methods of squashing out the cell contents in a staining and fixing medium, such as acetic acid containing orcein, as recommended by La Cour (**9**), were found to give satisfactory differentiation of the chromosomal material and to expedite efficiently the examination of the large number of sporophores necessary in this work.

In our method the gills of the agaric sporophores were dissected out on a drop of aceto-orcein on a slide, under a low power microscope. The debris was removed and a cover slip was placed in position. The slide was then warmed quickly over an alcohol flame. Excess aceto-orcein was removed with bibulous paper and the preparation was flattened and squashed out by carefully applying considerable pressure on the cover glass, so as to separate the basidia as much as possible. The slides were then sealed with vaseline and remained in good condition for 7 to 8 days, a period which allowed enough time for careful examination and for the necessary recording and illustrating of the nuclear figures.

The leuco-basic fuchsin method recommended by Darlington and La Cour (**3**) was used on several occasions to conform observations made with the aceto-orcein technique, but the latter was preferentially chosen for use throughout this work.

Photographic illustration of the nuclear behavior in the Agaricales has apparently never been attempted, probably because of the technical difficulties in photography resulting from the small size of the nuclear structures. However, in the following pages we have attempted to illustrate

the nuclear history of *Omphalia flavida* mainly with the use of photomicrographs, because of the advantages of this direct portrayal.

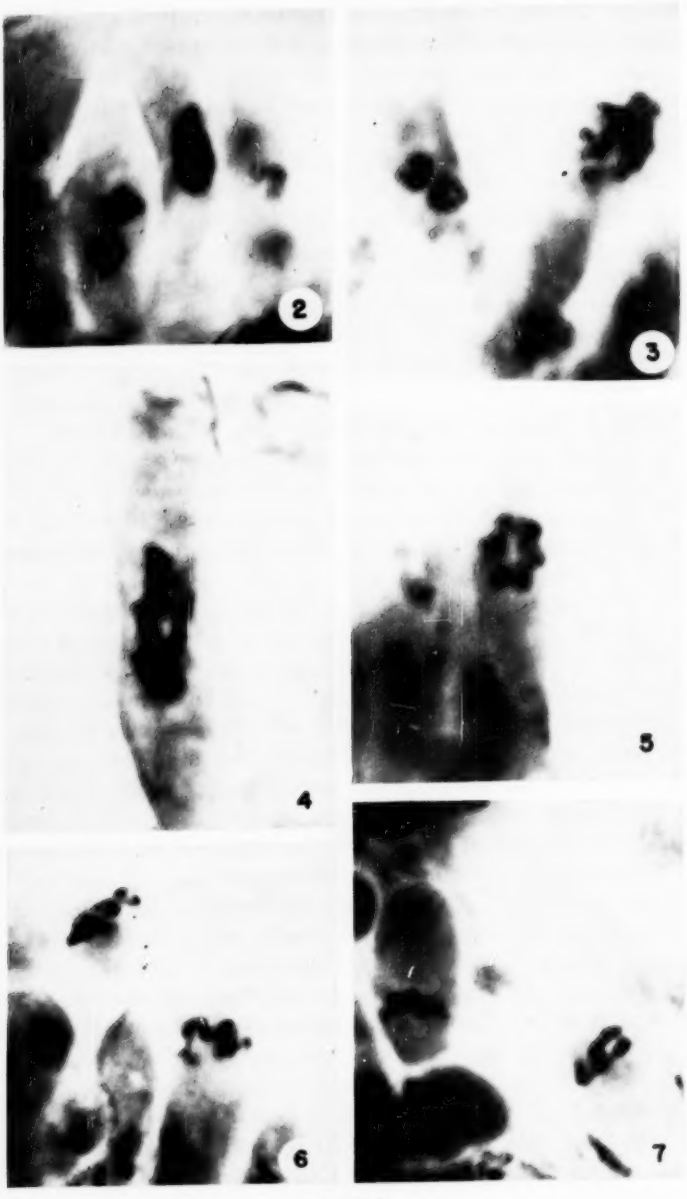
RESULTS

The mycelium of *Omphalia flavida* is of the typical basidiomycetous type, the cells being normally binucleate and presenting a clamp connection at every septum, a condition associated with the binuclearity of the cells.

The binuclear condition of the cells is maintained in the hymenium and all the young basidia present two nuclei, which are closely associated and arranged one above the other along the longitudinal axis of the basidium (FIG. 2). The nuclei in these early stages are about 1 to 1.4 μ in diameter and by the time the basidium has reached approximately one-half of its maximum size the nuclei begin to approach each other. The nuclei fuse and the resulting fusion nucleus swells and appears elongated parallel to the longitudinal axis of the basidium. This fusion nucleus now measures $3 \times 1.5\ \mu$ and occupies a larger portion of the basidium than that occupied by the two original nuclei, indicating an increase in total volume.

As the volume of the nucleus increases and it reaches approximately $2.5 \times 3.5\ \mu$, the individual chromosomes, made up of single long chromatids, become visible. These early chromosomes, probably representing leptotene stages of prophase, as in FIG. 3, are made up of a series of granules which stain darkly, connected with light staining regions.

The early prophase stages are usually of short duration and, as prophase advances, a gradual shortening and thickening of the chromosomes takes place (FIG. 4). Individual chromosomes, presumably bivalent, finally become grouped in an irregular mass at the center of the basidium in a region of clear nuclear sap and then move towards the periphery of the nucleus (FIG. 5). At a stage interpreted as diakinesis, six bivalents could be seen arranged on the inside of the nuclear membrane. As a result of the thickening of the chromosomes it is quite difficult to determine in this step the double nature of the chromosomes. Apparently, the nuclei pass through diakinesis very rapidly and it is only in a very few cases that one is able to find basidia fixed at this particular point. In *Omphalia flavida*, the ***n*** number determined at diakinesis is *6* and it agrees well with the chromosome number reported for several species of the genus *Mycena*, e.g. *M. haematopoda* Wakayama (**12**), the genus in which, as has been shown by Dennis (**4**), *Omphalia flavida* technically belongs.



FIGS. 2–7.

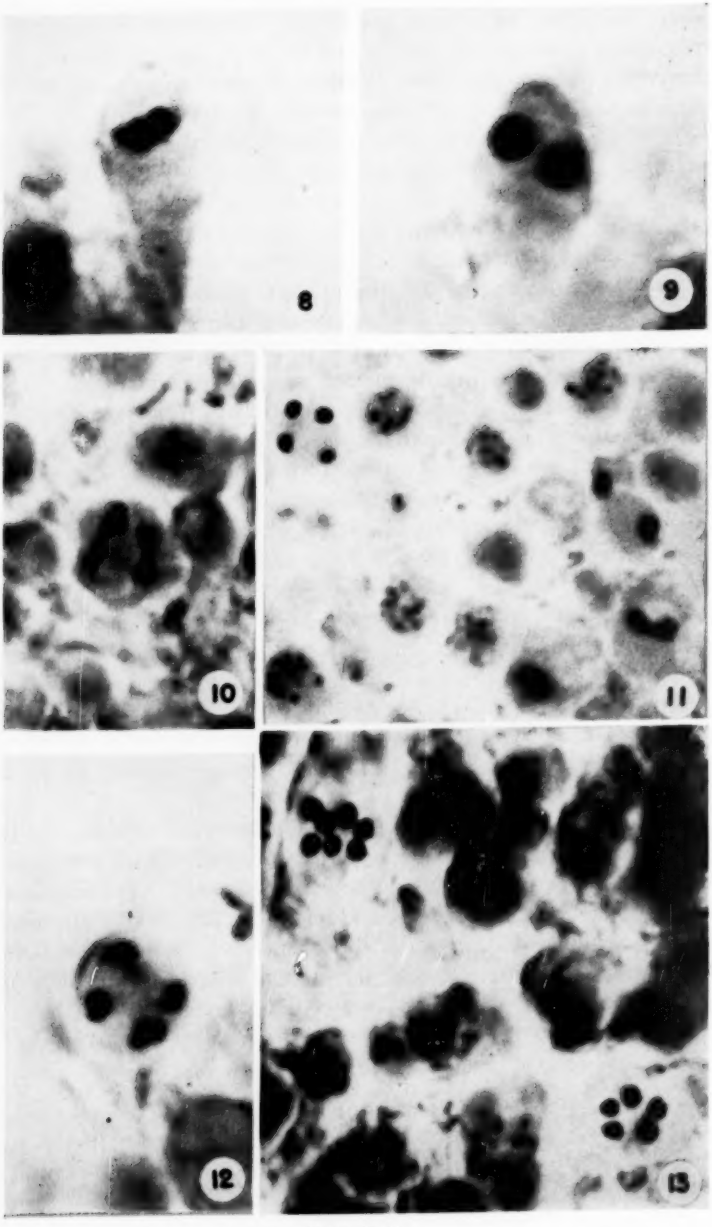
Very soon after diakinesis, the nuclear membrane disappears, the chromosomes contract still further and begin to arrange themselves on a transverse plate at the upper or distal third of the basidium. This metaphase plate is consistently transverse to the longitudinal axis of the basidium (FIGS. 6, 7). The chromosomes at this stage stain deeply with aceto-orcein, but the spindle elements are difficult to ascertain.

The chromosomes at anaphase become closely associated with one another during their migration, so that even at early intervals of this stage, one can no longer make out the individual chromosomes in each group as they advance towards the opposite poles. In the late stages of anaphase I, the repulsion and migration of the chromosomes towards the poles is followed by a period of elongation in the middle region of the spindle, in what is usually termed stem body formation (FIG. 8). By the time the chromosomes reach the poles, which are near the lateral walls at the tip of the basidium (telophase I), the spindle disappears and a nuclear membrane is formed around each group of chromosomes (FIG. 9). The nuclei are then seen to increase somewhat in volume in preparation for the second meiotic division. These nuclei, after disintegration of their membranes, pass directly into the prometaphase of the second division, a small spindle forms for each nucleus on the same transverse plane and the second meiotic division is established. The two nuclei divide almost simultaneously and, as in anaphase I, the heavy staining reaction of the spindle gives the impression of continuity between the chromosomes of each dividing nucleus (FIG. 10). The spindles formed at the second meiotic division are considerably smaller than the one formed at the first division and they are usually in parallel alignment.

At the end of meiosis, the four nuclei lie close to or touching the wall of the basidium, arranged almost equidistantly and still on a transverse plane in the upper third of the basidium (FIGS. 11, 12). From this point on, there is considerable variation in the behavior of the nuclei, associated with the characteristic third (mitotic) division that occurs. For convenience, we have divided the nuclear behaviors that follow into three patterns which we have called: typical, atypical A, and atypical B cycles.

In the typical case, which appears more consistently (60 to 75% of

FIGS. 2–7. Young basidia showing: 2. The early binucleate condition and a fusion nucleus. 3. Nuclei immediately preceding fusion and an early prophase nucleus. 4. An elongated early prophase nucleus. 5. Diakinesis. 6. The chromosomes at the metaphase plate. 7. Metaphase at the left and the first stage of anaphase I at the right.



FIGS. 8–13.

the basidia) in the cytological preparations than do the other types, the four resulting nuclei characteristically divide again to produce 8 nuclei, but these divisions do not occur simultaneously and usually the nuclei divide one after another. In rapid succession the four nuclei divide and the eight resulting nuclei lie in a limited region in the distal part of the basidium (FIG. 13). These divisions no longer occur, as in meiosis, in a single plane and for this reason it is extremely difficult, for purposes of photographic illustration, to find cases in which most of the eight nuclei can be seen in any one plane of focus. The nuclei resulting from these divisions measure about 0.6 to 0.7 μ in diameter.

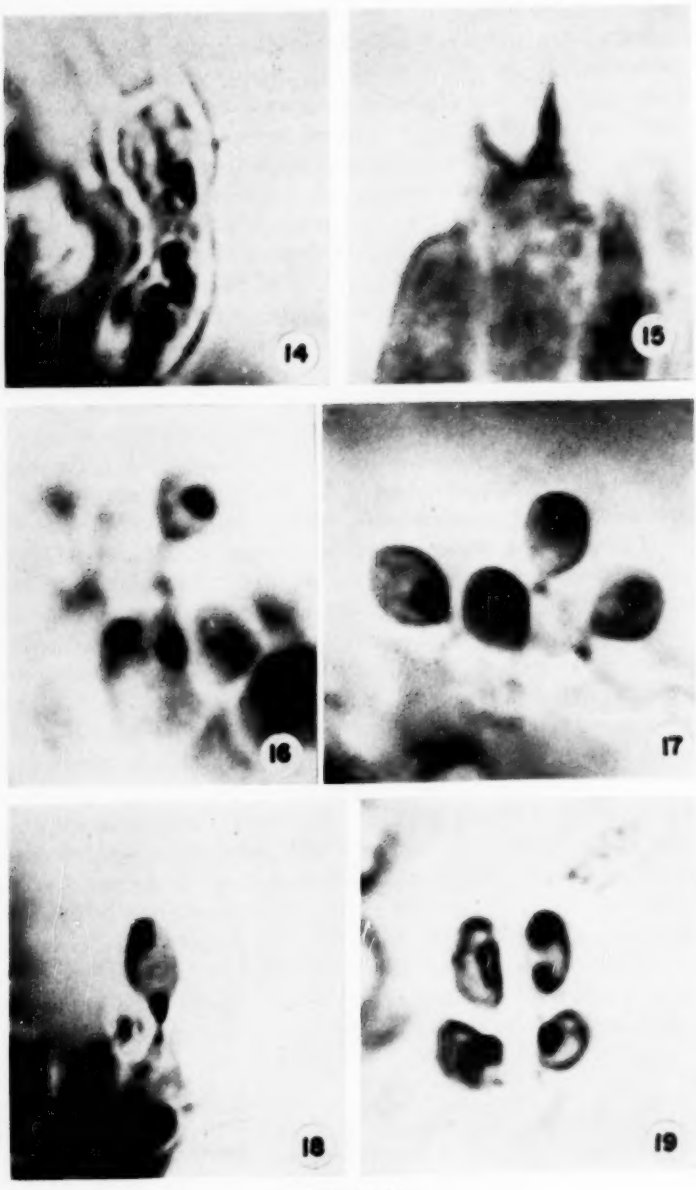
After this third division a stage of rather unusual behavior follows. The nuclei move down towards the basal part of the basidium and halt somewhere below the middle portion, where they elongate; this is accompanied by a loss in staining intensity.

As soon as the four sterigmata are fully formed, the eight nuclei again contract and they now appear scattered, one after the other, throughout the entire length of the basidium (FIG. 14). During this process the nuclei have increased considerably in volume, measuring now $1.5 \times 0.8\ \mu$. In this typical cycle, the first four nuclei to reach the top of the basidium arrange themselves at the base of the sterigmata. The time of migration of the nuclei into the sterigmata varies considerably; it may occur while there is still no sign of basidiospore formation or it may occur at the time when the spores are fully formed, or at intermediate stages between these two extremes. In any case, there seems to be no fixed requirement of spore formation to induce the elongation of nuclei which is necessary in order for them to pass through the narrow lumen of the sterigmata. FIGURES 15 and 16 illustrate the variability in form and behavior that characterizes the migration of nuclei through the sterigmata.

Upon reaching the basal portion of the spore, the elongated nucleus rounds up and migrates toward the distal end of the spore. Upon reorganization within the spore, the nucleus becomes not only homogeneous but also perfectly spherical in shape and, in a mature spore, it is found close to or touching the spore wall at the distal end (FIG. 17).

The migration of the four nuclei in this typical cycle results in a basidium, ready to discharge its four uninucleate spores, while in the

FIGS. 8–13. Intermediate stages in the development of the basidium showing: 8. Anaphase I. 9. Telophase I. 10. Anaphase II, top view. 11. At the upper left, the four nuclei resulting from meiosis; at the lower right, anaphase I and telophase I. Top view, × 4200. 12. Telophase II, top view. 13. At the upper left, a basidium with seven nuclei; at the lower right, a basidium with five nuclei. Top view.



FIGS. 14–19.

basal portion of this same basidium there still remain four residual nuclei. In any one cytological preparation it is easy to encounter a number of shrunken basidia which have discharged their spores and still show the four residual nuclei, as is the case in many other gilled fungi.

An alternate nuclear cycle, referred to, for convenience, as *atypical A* and occurring in 10 to 20% of the basidia in any one preparation, may be characterized as one consisting of double nuclear migration. In this cycle, the four nuclei that normally become residual do not remain behind but follow their companion nuclei very closely and enter the sterigmata right in back of them (FIG. 18). The first nucleus reorganizes at the distal end of the spore and the second at the basal end, so that the mature spores show two nuclei at opposite ends. These nuclei are never found closely associated within the spores.

Another alternate nuclear behavior (*atypical B*) involves a delayed third nuclear division. In about 10 to 20% of the basidia, the third division does not occur until after the four nuclei produced at meiosis have migrated into the spores. In this nuclear pattern, the four nuclei resulting from meiosis elongate and pass through the sterigmata as in the typical eight-nucleate basidium. Each nucleus rounds up and reorganizes in the center of the spore while the latter is still in the early stages of its development. The nucleus then increases in size, from about 1 μ to 1.5 μ in diameter, and divides in typical fashion, producing a small spindle and a stem body between the two groups of chromosomes, division always occurring along or at a slight angle to the long axis of the spore (FIG. 19).

The result of the two atypical cycles described above is a basidium bearing four binucleate spores and containing no residual nuclei, as is shown in FIG. 20 which summarizes diagrammatically the main nuclear cycles in this *Omphalia*.

The haphazard manner in which the third nuclear division occurs allows the possibility of a number of variant and intermediate patterns, which need be considered only briefly as they are of infrequent occurrence. For instance, one can find basidia bearing some uninucleate and some binucleate spores, containing also the appropriate number of residual nuclei which have remained behind in the basidium. Also, inter-

FIGS. 14–19. Late stages in the development of the basidium showing: 14. A basidium with six nuclei; two other nuclei present are out of focus. 15. Elongation of a nucleus within the sterigma. 16. A nucleus within a spore and two other nuclei arranged at the base of the sterigmata. 17. Four uninucleate spores belonging to the same basidium. 18. A spore undergoing double nuclear migration. 19. Four spores of a basidium; one is binucleate, one uninucleate, and two have nuclei in process of division. FIGS. 2–19 magnified $\times$ 4600, unless otherwise indicated.

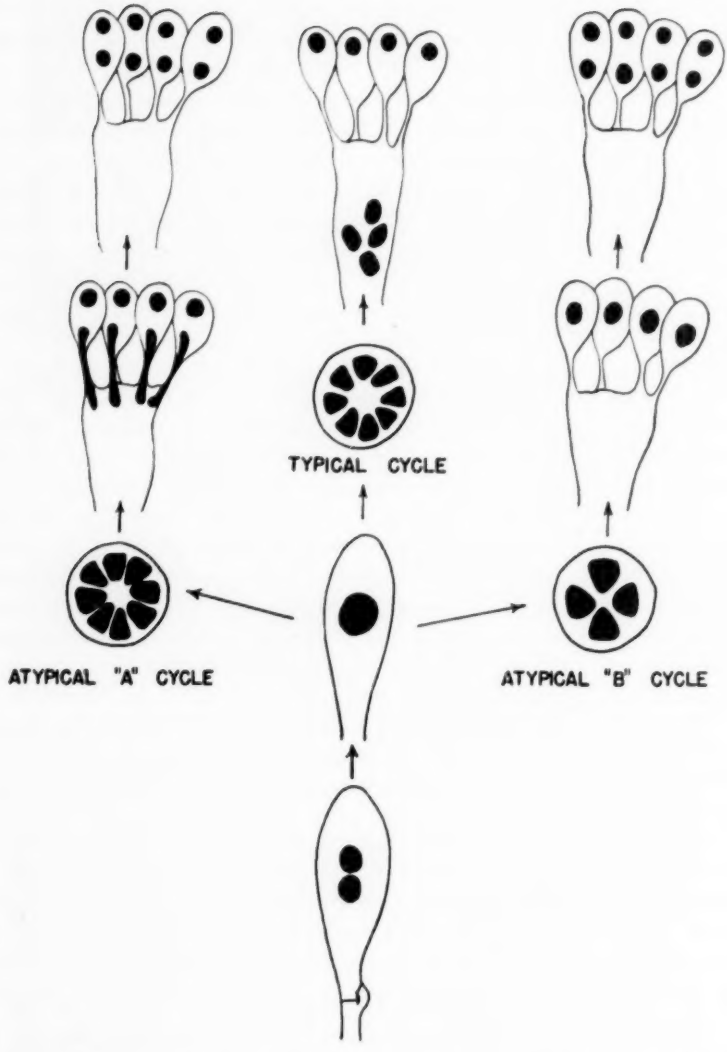


FIG. 20. Diagram showing the main nuclear cycles in the development of the basidia of *Omphalia flavida*.

mediate behaviors between the two atypical cycles have been observed and in these cases it would appear that in the same basidium one binucleate spore may have received its nuclei by double migration, whereas another spore may have received the two nuclei by delayed third division.

DISCUSSION

The nuclear behaviors described above are by no means new in the cytology of the Hymenomycetes, but what distinguishes *Omphalia flavida* as unique is the fact that all these alternate nuclear patterns occur in the same hymenial layer. All other gilled fungi, so far as the writer is aware, present a nuclear picture which is more or less constant and fixed for the same species and normally they do not depart from one particular pattern of behavior.

In most of the species of the genus *Mycena* and the genus *Cantharellus* the type of nuclear behavior which we have described as typical, producing four uninucleate spores and four residual nuclei per basidium, is the characteristic pattern (**6**, **11**). In *Clitopilus orcella* (**10**) and *Mycena murina* (**11**) the normal procedure is a nuclear double migration which occurs after the three divisions of the fusion nucleus, which we have described above as atypical cycle A.

The atypical cycle B in *Omphalia flavida*, characterized by a delayed third division, is the usual process in *Schizophyllum commune* (**5**), most Amanitas, Lepiotas, and many other advanced forms (**7**, **8**).

As far as could be determined from the literature, however, there is no report that any member of the Agaricales can present all three types of nuclear behavior, together with other intermediate cycles, as has been described in this study of *Omphalia flavida*. Several authors have stressed the fact that in this group of fungi the third division may be more or less variable, but the range of variation found in *Omphalia flavida* has not been reported in any of the species that have been studied.

Kühner (**7**, **8**) has pointed out that in the white-spored Agaricales there is a progressive tendency for the third division to be delayed until it finally takes place within the spore. All the primitive gymnocarpic four-spored forms show a number of primitive structural features, coupled with the formation of uninucleate spores and the retention of four residual nuclei in the basidium, whereas advanced angiocarpic forms have a high degree of structural differentiation and, associated with it, they invariably have binucleate spores and no residual nuclei. Thus, in advanced forms all eight nuclei are utilized, whereas in primitive forms only four are used, the remaining four being left as residual nuclei. Considering the results presented in this paper, it would appear that *Omphalia flavida*

occupies a very neat position in Kühner's evolutionary scheme having: 1) primitive characteristics in the formation of uninucleate spores and four residual nuclei, as in the typical cycle; 2) some evolutionary advances for the utilization of the nuclei which normally remain residual, i.e., double migration and delayed third division, as in the atypical cycles we have described above.

The variability of the nuclear behavior in *Omphalia flavida* poses some interesting questions in regard to sexual phenomena in this species. In preliminary experiments on basidiospore germination the writer has found that this fungus is normally heterothallic, each basidiospore on germination giving rise to a primary mycelium with no clamp connections, and it is clear that spores of this type could be produced in all cases in the typical and atypical B cycles. However, in a few cases we have been able to determine the sporadic formation of diploid, clamp-bearing mycelium of monosporous origin and it would appear that homothallic spores of this type could be produced in the atypical cycle A, when two nuclei of different sex potentialities migrate into the spore.

Further genetic work is needed to obtain conclusive proof of the homo-heterothallism of *Omphalia flavida* and investigations have been started along these lines. It is hoped that the genetic evidence obtained will have definite bearing on the scheme suggested by the cytological work.

SUMMARY

The application of the aceto-orcein squash technique for cytological studies of nuclear behavior in *Omphalia flavida* has brought out the following details:

1. The basidium of *Omphalia flavida* contains two nuclei which fuse in the early stages of development. Typically, the fusion nucleus undergoes three divisions, two of which are meiotic. All these divisions occur transversely to the long axis of the basidium. At diakinesis the chromosome number was determined, the haploid number being six.

2. The third nuclear division is variable and can occur either in the basidium or in the spores. In the typical cycle, this division occurs in the basidium and, after a resting period, four nuclei migrate into the spores and four remain as residual nuclei in the basidium.

3. In 10 to 20% of the basidia, two nuclei migrate to each one of the spores in a cycle designated as atypical A, characterized by migration of all of the nuclei.

4. Also in 10 to 20% of the cases, the third division occurs in the spores, this cycle being characterized by delayed third division and designated as atypical B.

5. A number of intermediate cycles are also present and it is clear that *Omphalia flavida* is distinctive in having so many alternate nuclear behaviors.

Cytologically this organism occupies a neat intermediate position in Kühner's evolutionary scheme for the white-spored Agaricales. The variability in nuclear behavior gives rise to the interesting situation that this organism may be either homothallic or heterothallic in its sexuality depending on the number and nature of the nuclei in its spores.

ACKNOWLEDGMENTS

The writer is deeply indebted to Professor W. H. Weston for his constant interest and encouragement throughout the course of this investigation and his help during the preparation of this manuscript. Grateful acknowledgment is also made to Dr. C. M. Wilson and Dr. F. L. Wellman for their kind and helpful advice on many aspects of this work.

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FURTHER NOTES ON AMYLOID TRAMAL HYPHAE IN GOMPHIDIUS

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In recent years there has been a tendency to consider that the genus *Gomphidius* was not closely related to *Hygrophorus* as we previously supposed, and to consider it close to the boletes instead. However, there is general agreement that it is a peculiar and isolated genus. In such groups as this the discovery of new characters is of particular interest, but their evaluation often requires considerable study over a period of time in order to ascertain the limits of applicability. Eventually, however, we arrive at a better understanding of the fungi and can circumscribe species and genera more accurately.

The junior author, in the course of a routine study of mycorrhizal fungi, noticed, when studying certain species of *Gomphidius* (those in subgenus *Chroogomphus*), that the trama of the gills and pileus when mounted in Melzer's reagent were so dark that all detail was obscured, in contrast to the usual clearing effect of this reagent. He consulted the senior author in regard to the situation, who immediately recognized it for what it was—a strong positive amyloid reaction of the hyphal walls and incrusting material. With this information as a start, we surveyed the herbarium specimens of species in this genus as far as material was available with the following results. The specimens used were those checked by Singer in the course of monographing the genus (Singer, Mycologia **41**: 462–489. 1949).

1. GOMPHIDIUS TOMENTOSUS Murrill. Pileus trama and cuticle blackish violet and with violet incrusting granules and debris adhering to the walls. The hyphal walls individually dark violet. The gill trama was violet throughout including the subhymenium. The humenium was orange to yellow but finally some basidia showed amyloid granules but the reaction was always weak. The cystidia were yellow to orange with no tendency to blue noted.

2. GOMPHIDIUS LEPTOCYSTIS Singer. Pileus cuticle and trama yellow, or with only scattered amyloid hyphae present in crushed mounts. Amy-

[1] Papers from the University of Michigan Herbarium, the Department of Botany, and the School of Natural Resources, University of Michigan, Ann Arbor, Michigan. Published out of order at the expense of the University of Michigan Herbarium.

loid granules were also present in some hyphae. The gill trama was not amyloid, or, if the mount was crushed, a few hyphae could be found with bluish walls but the reaction was weak. In the hymenium the basidia were not amyloid or contained only a few dark granules. In crushed mounts, most of the cystidia gradually developed pale blue walls, but the reaction was erratic.

The above data were taken from the type. In other collections erratic results were obtained, but the reactions were generally weak and served easily to distinguish collections from *G. tomentosus*. Fresh material should be studied, but since the specimens examined were well preserved, the erratic results could not be attributed to poor drying.

3. GOMPHIDIUS VINICOLOR Peck. Here the viscid pellicle was strongly violaceous as was also the pileus trama made up of broader non-gelatinous hyphae. The gill trama was also strongly violaceous in the central strand but the flanking tissue was yellow. In the hymenium the apices of many of the basidia were bluish. The cystidia were yellow in the interior and with hyaline walls, but the walls in the lower portion of the cystidium, where they were thickest, slowly became pale blue to dingy grayish violet. This change was observed in one-fourth to one-half of the cystidia in a crushed mount.

4. GOMPHIDIUS ALACHUANUS Murrill (*G. vinicolor* ssp. *jamaicensis* in Singer). The collection studied was made by Murrill in 1939 in Florida. The pellicle is pale yellow, the pileus and gill trama are dark violet, the hymenium yellow and in crushed mounts the cystidia show only a very weak amyloid change in the thickened part of the wall.

5. GOMPHIDIUS OCHRACEOUS Kauffman. The pellicle is mostly of yellow narrow hyphae but some of them slowly turn blue to dark violet, and amyloid granules finally develop in some hyphae in which the wall apparently does not change. The body of the pileus trama becomes dark violet, as do the subhymenium and gill trama. The hymenium is yellowish, but young basidia with weakly amyloid walls and some amyloid content as granules were seen. The cystidia were not amyloid; both walls and content remained yellow in iodine.

6. GOMPHIDIUS SUPERIORNESIS Kauff. & Smith. The pellicle remained yellow, the pileus trama became dark violet but the yellow content of the cells tended to obscure the reaction. The gill trama and subhymenium were violet to bluish gray. The hymenium was pale yellow at first but slowly turned bluish gray, especially in the pedicels and young basidia. The cystidia did not react.

7. GOMPHIDIUS RUTILUS (Fr.) Lundell (the type of *G. viscidus* var. *columbiana* Kauff. was studied. Singer considers this typical *G. ruti-*

lus). The pellicle remained orange yellow, the pileus trama turned dark bluish gray, often with a bister tone before the mount was crushed, but individual hyphae were seen to have clear violet walls. The gill trama and subhymenium were bluish gray. The hymenium, including the cystidia, remained yellow to orange yellow.

8. GOMPHIDIUS FLAVIPES Peck. Pileus trama and cuticle orange brown but central portion of the trama slowly violaceous brown and this change extending down into the gill trama. Gill trama with a violaceous-brown central strand gradually becoming violaceous gray if crushed out; flanking tissue on either side (remainder of gill trama) yellow. Hymenium entirely yellow to orange yellow, including the cystidia.

9. GOMPHIDIUS SUBROSEUS, G. GLUTINOSUS, G. OREGONENSIS, and G. MACULATUS. No positive reaction with Melzer's reagent was obtained in any part of the fruiting body in the material examined. However, in the light of Imler's work (see discussion), all of these should be checked again from fresh material.

DISCUSSION

A survey of the literature has shown that Imler (Bul. Soc. Myc. Fr. **66**: 201–202. 1950) studied this reaction for a few species and pointed out that it was positive in *G. viscidus*. He also indicated some positive reaction on the base of the stipes for *G. glutinosus*.

From the work of others and from the present study a number of important points can be made:

1. A very careful and thorough study should be made of all Gomphidii in the fresh condition to get more information on this important character. The results obtained so far are not in entire agreement. In some species they have been found erratic. For instance, why does the character show up best in mounts of tissue *crushed* in Melzer's solution? In addition there are very likely important differences in the degree of the reaction, such as those between *G. tomentosus* and *G. leptocystis*, which can be used in conjunction with other characters to delimit species more clearly. The results of Imler for *G. viscidus* and ours on *G. rutilus* (*G. viscidus* var. *columbiana*) are not in agreement. They probably indicate a taxonomic difference between the two taxa. Singer treated Kauffman's variety as typical *G. rutilus*. In any event, the application of this test needs to be standardized so that results of various investigators are directly comparable.

2. On the basis of the iodine reaction, *Laricogomphus* appears to be an artificial group. *G. flavipes* should be removed to *Chroogomphus*.

3. The idea of the relationship of the Gomphidii to the boletes is strengthened by the presence of species in both groups which show varying degrees of positive reaction of the tramal tissues and other elements.

4. The matter of the nomenclature of reactions with Melzer's reagent becomes increasingly important as more fungi are found in which the reactions are important. At the present time the term amyloid is being used in a number of ways. In order to stabilize and clarify usage we propose the following:

a) *Amyloid* to indicate a violet-black, violet, blue, grayish blue or pale gray color as the result of application of Melzer's solution.

b) *Pseudoamyloid* to indicate the brown, reddish brown, red to purple-red reactions, as in the hyphae of *Mycena* and the spores of the Lepiotaceae. In the past this reaction has been called amyloid on the hyphae of *Mycena* and pseudoamyloid on spores of *Lepiota*.

c) *Non-amyloid* to apply to no reaction or one in the yellow to pale orange series.

5. The results of this study indicate that *G. tomentosus* would be favorable for more detailed chemical studies on the nature of the material which takes the blue reaction. Here incrusting particles, as well as the walls themselves, and granules in the cells, are all amyloid to the same degree.

6. One of the points involved in a further clarification of the meaning of this character in the Hymenomycetes is the emphasis to be placed upon it at the generic level. Perhaps *Chroogomphus* should be given generic rank. We feel that it would be premature to make such a change before a careful study of fresh material is made. From the work of Imler (l.c.) on boletes, it would not appear to have generic value in that group, but will help a great deal in clarifying species concepts.

7. It is interesting to note that the species of *Gomphidius* showing a positive reaction are mostly American: *G. tomentosus*, *G. leptocystis*, *G. vinicolor*, *G. ochraceus*, *G. superiorensis* and *G. flavipes*. *G. rutilus* is European.

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PERENNIAL POLYPORES OF NORTH AMERICA II. FOMES WITH COLORED CONTEXT, HYALINE SPORES

JOSIAH L. LOWE

(WITH 1 FIGURE)

A part of the North American species of *Fomes* in the family Polyporaceae, the species with colored context and colored spores, have already been treated taxonomically (Lowe, **1952**). This paper considers species of *Fomes* which are morphologically similar except that they have hyaline spores. These have been placed in the genera *Pyropolyporus, Porodaedalea, Melanoporus,* and *Elfvingia* or *Elfvingiella* in part, by Murrill (**1907–08**; **1914**; **1915 a**, **b**, **c**).

Seven of the hyaline-spored species (i.e., *Fomes conchatus, F. fomentarius, F. fulvus, F. igniarius, F. pini, F. robustus,* and *F. tenuis*) are widespread in Europe and America, and their identities conform with the concepts current in European and American mycology. The concepts of the remaining species are based on a study of type material and other specimens as cited. The microscopic characteristics were obtained by study of free-hand sections mounted in 2 percent potassium hydroxide solution.

The species treated do not form a homogeneous group. *Fomes alboincarnatus* and *F. melanoporus* are each unique. *F. fomentarius* and *F. marmoratus* on the other hand seem to be closely related, and form a group distinct from all the rest. The remainder of the species agree in many characteristics but individually are quite distinct from each other except for *F. igniarius* and *F. fulvus.* These are so alike that the latter appears to be a variant brought about by its peculiar substratum.

This paper considers 59 species and recognizes 17. The following are reduced to synonomy for the first time:

Pyropolyporus cinchonensis = *F. roseocinereus*
Pyropolyporus crustosus = *F. robustus*
Pyropolyporus haematoxyli = *F. robustus*
Pyropolyporus robinsoniae = *F. zealandicus*
Pyropolyporus texanus = *F. robustus*
Trametes gausapata = *T. carbonaria*

Fomes baccharidis and *F. inflexibilis*, which have been reported in the flora of North America, apparently do not occur in the area.

The distribution of common, widespread species is adapted from Overholts (**1953**). The range of the rarer species is based on cited specimens, the loan of which is gratefully acknowledged and is indicated as follows: the mycological collections of the Bureau of Plant Industry and the Smithsonian Institution at Beltsville, Maryland, by (BPI); the Farlow Cryptogamic Herbarium at Cambridge, Massachusetts, by (FH); the Royal Botanic Gardens at Kew, England, by (K); the New York Botanical Garden at New York City, by (NY); and the Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, at Paris, France, by (PC); all other herbaria are not abbreviated. A part of the work was done at Beltsville as collaborator of the Division of Mycology and Disease Survey, U. S. Department of Agriculture.

KEY TO THE SPECIES

1a. Context orange to brick red.....*F. albomarginatus*
1b. Context dark purplish-brown.....*F. melanoporus*
1c. Context yellowish-brown or reddish-brown
  2a. Pileus with a thick crust and soft fibrous context
    3a. Pores about 3 per mm; spores 17–20 × 5–6 μ.....*F. fomentarius*
    3b. Pores 4–6 per mm; spores 8–13 × 3–4 μ.....*F. marmoratus*
  2b. Pileus usually without a thick crust; context mostly firm to hard
    4a. Spores cylindrical
      5a. Pores 5–6 per mm; setae 25–32 μ long; spores 5–6 × 2–2.5 μ *F. nigrolimitatus*
      5b. Pores 2–4 per mm; setae 25–55 μ long; spores 5–9 × 1.5–2 μ..*F. tenuis*
    4b. Spores oblong-ellipsoid to globose
      6a. Setae when present usually ventricose, rarely exceeding 20 μ in length, often rare or lacking
        7a. Context bright yellowish-brown; spores often up to 8 μ in diameter; basidia 9–11 μ in diameter.....*F. robustus*
        7b. Context dull yellowish- or reddish-brown; spores up to 6 μ in diameter; basidia not over 7.5 μ in diameter
          8a. Spores 2–4 μ broad; context usually soft
            9a. Setae present; associated with a conspicuous pocket rot of the wood of coniferous trees in the Pacific Northwest.....*F. repandus*
            9b. Setae absent; known only from the West Indies *F. subflexibilis*
          8b. Spores 4–5 μ broad; context firm to hard
            10a. On a variety of substrata, the sporophore often large *F. igniarius*
            10b. Restricted to trees bearing stone fruits, the sporophore small.....*F. fulvus*

10b. Setae usually subulate, usually exceeding 20 μ in length
11a. Some and often most of setae with curved apices..*F. zealandicus*
11b. All of setae with straight apices
12a. Pores 1–4 per mm
13a. Spores 4–5 μ in diameter; on the wood of coniferous trees, associated with a white pocket rot....*F. pini*
13b. Spores 5–6 × 4.5–6 μ; on the wood of deciduous trees, associated with a uniform soft white rot
F. occidentalis
12b. Pores 4–8 per mm or smaller
14a. Spores 4–5 μ wide; hyphae 2–3 μ in diameter
F. conchatus
14b. Spores 2–3 μ wide; hyphae usually 3–5 μ in diameter
15a. Spores ellipsoid, 4.5–5 × 2.5–3 μ; pores 5 per mm.....*F. taxodii*
15b. Spores broadly oval, 3.5–4 × 2–3 μ; pores 6–10 per mm.....*F. roseocinereus*

ANNOTATED LIST OF SPECIES

abramsianus Murr. (*Pyropolyporus*). A synonym of *F. robustus*. (NY-type; BPI-isotype.)

albomarginatus (Zipp ex Lév.) Sacc. (*Fomes*). Rarely resupinate or effused-reflexed, usually sessile, the pileus applanate, usually reddish; pore surface often with a paler, wide, sterile margin, the pores 6–8 or rarely to 10 per mm; context orange to brick red; tubes in very distinct layers; spores not found in the type collection, very probably smooth, oblong, about 3 × 1.5 μ. On wood in British Honduras (NY); a frequent species in the East Indies, and known from Africa. *Polyporus kermes* is a synonym. (PC-type; BPI-isotype.)

arctostaphyli Long (*Fomes*). A depauperate form of *F. igniarius*. (BPI-type.)

baccharidis (Pat.) Lloyd (*Fomes*). Reported by Murrill (**1915b**, p. 80) from Guadeloupe but specimen not found. The fungus is known only from Ecuador. (FH-type; NY- and BPI-isotypes.)

bakeri Murr. (*Pyropolyporus*). A synonym of *F. robustus*. (NY-type; BPI-isotype.)

betulina Murr. (*Fomitiporella*). Name applied to the resupinate condition of *F. igniarius*. (NY-type; BPI-isotype.)

borealis Lloyd (*Fomes*). A synonym of *F. igniarius*. (BPI-type.)

calkinsii Murr. (*Pyropolyporus*). A synonym of *F. robustus*. (NY-type; BPI-isotype.)

cinchonensis Murr. (*Pyropolyporus*). A synonym of *F. roseocinereus*. (NY-type; BPI-isotype.)

conchatus (Pers. ex Fries) Gill. (*Fomes*). Often effused-reflexed, or sessile, tomentose, in age glabrous and sometimes thinly incrusted; pores 4–7 per mm; context reddish-brown, woody, with one to several black lines, the hyphae thick-walled, nonseptate, 2–3 μ in diameter; setae broadly subulate to ventricose, projecting up to 20 μ, 18–30 × 5–10 μ; spores ovoid to subglobose, 4.5–6 × 4–5 μ. On wood of deciduous trees from New Brunswick west to Manitoba and southward to Florida and Texas; associated with a rusty white rot. *Fomes densus* is a synonym. *F. pini* resembles it but has larger pores, and *F. zealandicus* is also very similar, but differs in having curved setae.

crustosus Murr. (*Pyropolyporus*). A synonym of *F. robustus*. Murrill (**1907–08**, p. 104) reduces his species to synonomy under *F. inflexibilis*, but *P. crustosus* differs from the type specimen of *F. inflexibilis* in having larger pores, and larger, septate hyphae. (NY-type.)

densus Lloyd (*Fomes*). A synonym of *F. conchatus*. Lloyd's specimens are thick, heavy forms of this species, as previously stated (Lowe, **1952**, p. 231). (BPI-paratypes.)

elatus Lév. (*Polyporus*). Type from Guadeloupe; specimen not at Paris.

fasciata (Swartz ex Fries) Murr. (*Elfvingia* or *Elfvingiella*). The name is of uncertain application to the fungus here recognized as *F. marmoratus*.

fomentarius (L. ex Fries) Kickx (*Fomes*). Ungulate, with a thick glabrous crust; pores about 3 per mm; context soft-fibrous, yellowish-brown; setae none; spores hyaline, smooth, oblong to cylindric-ellipsoid, 17–20 × 5–6 μ. On dead trees or occasionally on wood of living trees from Newfoundland south to Tennessee, westward to the prairies, and from Montana to Washington northward to Alaska; associated with a white mottled rot. It is similar to *F. marmoratus* but with larger pores and larger spores.

fulvus (Scop. ex Fries) Gill. (*Fomes*). Usually at first resupinate, by annual growth forming a subcircular to elongated pendent pileus, rarely sessile and convex to ungulate, up to 3 × 10 × 5 cm; pores 4–6 per mm; context yellowish-brown; tubes occasionally white-stuffed; setae ventricose, 10–16 × 6–8 μ; basidia 5.5–6.5 μ in diameter; spores oval to subglobose, 4–5 × 4–4.5 μ. On wood of drupaceous hosts from Maine and Ontario to Georgia, westward to the prairies, and from New Mexico north to Montana and Manitoba; associated with a soft white rot. *Boletus pomaceus* is a synonym. The species seems to be scarcely more than a form of *Fomes igniarius*, dwarfed because of its occurrence on small branches.

gausapata Berk. & Rav. (*Trametes*). Type specimen (Ravenel *1327*) as designated by Miss Wakefield (Trans. Brit. Myc. Soc. **22**: 185. 1938) is *Trametes carbonaria* (Berk. & Curt.) Overh.; another American specimen (Ravenel *2922*) is *Irpex farinaceus* Fries. (K-type.)

gilvoides Lloyd (*Trametes*). This fungus, placed in synonomy with *F. tenuis* by Overholts (**1953**, p. 67), appears to be a distinct species. (BPI-type.)

haematoxyli Murr. (*Pyropolyporus*). A synonym of *F. robustus*. (NY-type; BPI-isotype.)

hartigii Allesch. & Schn. (*Polyporus*). Name applied to western forms of *F. robustus* on the wood of coniferous trees; usually considered a synonym of *F. robustus*. (NY-type exsiccatae material.)

hyperboreus Berk. (*Polyporus*). Said to be from Arctic North America. Type not at Kew. Often considered a synonym of *F. igniarius*.

igniarius (L. ex Fries) Kickx (*Fomes*). Resupinate to applanate or ungulate, often becoming rather large and rimose in age; pores 4–5 per mm; context dark brown; old tubes usually conspicuously white-stuffed; setae ventricose, 11–14 × 5–8 μ; basidia 6.5–7.5 μ in diameter; spores ovoid to subglobose, 4–6 × 4–5 μ. On wood of living deciduous trees or on down logs throughout the United States, Canada, and Alaska; associated with a destructive soft white heartrot. *Fomes arctostaphyli, F. borealis, F. nigricans, Fomitiporella betulina,* and *Polyporus laevigatus* are synonyms.

inflexibilis (Berk.) Cooke (*Fomes*). Type specimen from Brazil (Spruce *52*) and paratype (Spruce *181*) are different from any fungus yet known in the North American flora. Murrill's determinations from Jamaica are here transferred to *F. robustus*. (K-type, paratype; NY- and BPI-isotypes.)

isabellina (Fries) Overholts (*Poria*). A name applied to resupinate specimens of *F. tenuis*.

kermes Berk. & Broome (*Polyporus*). A synonym of *F. albomarginatus*. (K-type; BPI-isotype.)

laevigatus Fries (*Polyporus*). Name applied to resupinate or subresupinate conditions of *F. igniarius*.

marmoratus (Berk. & Curt.) Cooke (*Fomes*). Applanate to ungulate, with a thick glabrous crust; pores 4–6 per mm; context soft-fibrous, dark brown; setae none; spores hyaline, oblong to subfusiform, 8–13 × 3–4 μ. On living trees from North Carolina to Texas and widespread southward in the tropics; associated with a white rot. *Polyporus sclerodermus* is a synonym. *Fomes fomentarius* differs primarily in the

size of its spores. Unfortunately *F. marmoratus* is very often sterile; the spore size here given is based on relatively few measurements. (K-type; FH-isotype.)

melanoporus (Mont.) Cooke (*Fomes*). Effused-reflexed to sessile, applanate to convex, dark purplish brown throughout; pores 6–8 per mm; setae none; spores smooth, oblong-ellipsoid to ellipsoid, 4–5 × 2.5–3 μ. On wood from Florida (NY), Nicaragua (NY; BPI), British Honduras (NY; BPI), Panama (NY), Jamaica (NY), Cuba (PC-type; BPI-isotype; NY), and Trinidad (NY); throughout the tropical areas of the world.

nicaraguensis Berk. & Curt. (*Polyporus*). Type specimen not found, and identity unknown.

nigricans (Fries) Cooke (*Fomes*). Name often applied to the more narrowly sulcate black forms of *F. igniarius*.

nigrolimitatus (Rom.) Egeland (*Fomes*). Resupinate and often widely effused to sessile; context reddish-brown, soft-punky, with one or more fine black lines; pores 5–6 per mm; setae broadly subulate, 25–32 × 6–9 μ; spores smooth, cylindrical, 5–6 × 2–2.5 μ. On wood of coniferous trees from Colorado and California northward to British Columbia and Alaska; associated with a conspicuous white pocket rot. *F. putearius* is a synonym.

novae-angliae Berk. & Curt. (*Polyporus*). Type is a single small sterile sporophore unlike any known from temperate North America. Conidiospores along the hymenium are of the type found on tropical material only. It seems likely that the specimen actually came from the tropical region, and that the type locality designated, "New Engl.," is an error. (K-type.)

occidentalis Overh. (*Fomes*). Resupinate to effused-reflexed, rarely sessile, at first pubescent, becoming glabrous; pores 3–4 per mm; context dark reddish-brown, the hyphae 3–5 μ in diameter; setae infrequent, subulate to ventricose, 35–50 × 6–12 μ; spores subglobose to globose, 5–6 × 4.5–6 μ. Known only on *Crataegus douglasii* Lindl. in Idaho; associated with a uniform soft white rot. (Pa. State College-isotype; BPI.)

piceinus Peck (*Polyporus*). The annual form of *F. pini*. (N.Y.S. Museum-type; NY- and BPI-isotypes.)

pini (Thore ex Fries) Karst. (*Fomes*). Sessile or in the annual form varying to narrowly effused-reflexed, conchate to ungulate, tomentose in zones when young, glabrous in age; pores 1–4 per mm, often somewhat daedaloid; context yellowish-brown or reddish-brown; setae subulate, 30–45 × 7–11 μ; spores hyaline or in age brownish, smooth,

subglobose to globose, 4–5 × 4–4.5 μ. On wood of coniferous trees throughout the United States, Canada and Alaska; associated with a destructive white pocket rot. *Cryptoderma yamanoi, Daedalea vorax,* and *Polyporus piccinus* are synonyms.

pomaceus Pers. (*Boletus*). A synonym of *F. fulvus.*

putearius Weir (*Fomes*). A synonym of *F. nigrolimitatus.* (BPI-type.)

repandus Overh. (*Fomes*). Resupinate or narrowly reflexed, soon blackish and hardened above; pores 5–7 per mm; tubes fragile; context usually soft, reddish-brown; setae few, broadly subulate to somewhat ventricose, 16–19 × 8–11 μ; basidia 9–12 × 5.5–7 μ; spores broadly ellipsoid to subglobose, 4.5–5 × 3–4 μ. On wood of coniferous trees in Montana, Idaho, and British Columbia; associated with a conspicuous white pocket rot. (Pa. State College-type.)

rhabarbarinus Berk. (*Polyporus*). Reported from Cuba as a synonym of *F. senex.* The type material was selected from Hooker's collection as the "only specimen . . . to which the description would apply." The type locality is unknown, although "probably from Brazil"; collections from North America are unknown. (K-type.)

rheicolor Lloyd (*Fomes*). Reported by Overholts from Puerto Rico; in his manual (Overholts, **1953**, p. 416) the species is placed in synonomy with *Polyporus porrectus* (Murr.) Sacc. & Trott.

robinsoniae Murr. (*Pyropolyporus*). A synonym of *F. zealandicus.* (NY-type; BPI-isotype.)

robustus Karst. (*Fomes*). (FIG. 1, a–c.) Resupinate, effused-reflexed, or sessile, smooth to deeply cracked above, often with a broad sterile marginal band; pores 4–6 per mm; context bright yellowish-brown, the hyphae septate, 3–7 μ in diameter; setae none; basidia 9–11 μ in diameter; spores subglobose, 5–8 μ in diameter. On hardwoods or conifers throughout the United States, in Quebec, British Columbia, Alaska, Mexico (BPI), Nicaragua (NY), and Jamaica (NY); associated with a soft white rot. *Polyporus hartigii, Pyropolyporus abramsianus, P. bakeri, P. calkinsii, P. crustosus, P. haematoxyli,* and *P. texanus* are synonyms. Resupinate forms have been treated under the form name *Poria punctata* (Lowe, **1948**, p. 167) and the following species proposed by Murrill were considered synonyms: *Fomitiporia dryophila, F. carleae, F. jamaicensis, F. laminata, F. langloisii, F. lloydii, F. maxoni, F. obliquiformis, F. tsugina,* and *Fuscoporia juniperina.*

roseocinereus (Murr.) Sacc. & Trott. (*Fomes*). Sessile, convex to ungulate, glabrous; pores 6–7(–10) per mm; context yellowish- or reddish-brown, the hyphae 3–5 μ in diameter; setae subulate, 20–30 ×

4–8 μ; spores broadly oval, 3.5–4 × 2–3 μ. On wood in Panama (NY), Costa Rica (NY), Cuba (NY-type; BPI-isotype), and Jamaica (NY; BPI). *Pyropolyporus cinchonensis* is a synonym.

sclerodermus Lév. (*Polyporus*). Type from Guadeloupe is sterile; surely a synonym of *Fomes marmoratus*. (PC-type.)

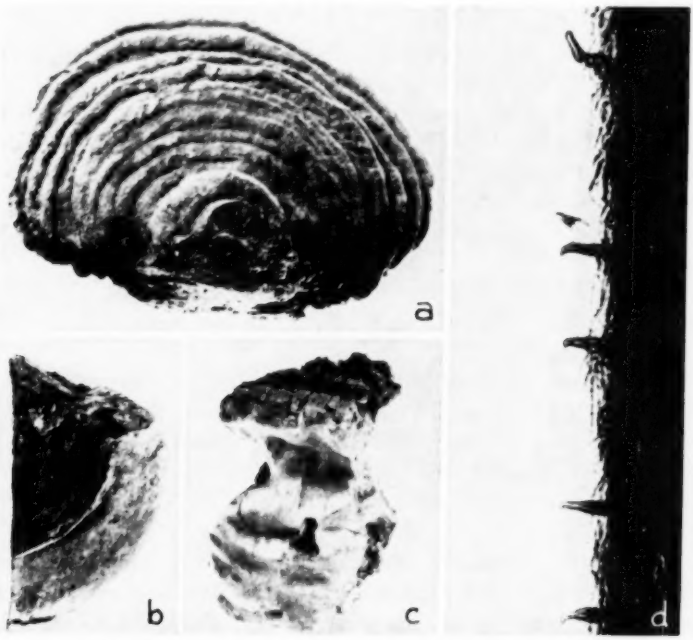

FIG. 1. a–c. Variations in the upper surface character of *Fomes robustus*. a. Smooth heavy crust of type material of *Pyropolyporus calkinsii*, the form most frequent in the southeastern United States. (× 0.7.) b. Broad paler margin in type of *P. bakeri*, the form frequent in the Northeast. (× 0.5.) c. Strongly rimose surface frequent in arid regions. Specimen from Durango, Mexico, March 1939, collected by E. O. Matthews. (BPI, × 0.4.) d. Hymenial features of paratype of *Fomes zealandicus* (*310*), showing curved apices of setae. (× 200.)

sclerodes Berk. (*Polyporus*). Type and paratypes from Cuba are not at Kew; apparently authentic material at the Farlow Herbarium is sterile and its relationships to the species here treated are unknown. (FH-here designated as lectotype.)

scleromyces Berk. & Curt. (*Polyporus*). Type and isotypes are sterile. Some synonyms have already been published (Lloydia **10**: 47. 1947) and Bresadola has annotated the type sheet "Vix differt a *Fomite semitosto* Berk." (K-type; FH-, NY-, and BPI-isotypes.)

senex (Nees & Mont.) Cooke (*Fomes*). The identity of this species is not yet satisfactorily established. No North American specimens have been seen which agree with the type. (PC-type; NY- and BPI-isotypes.)

setosus Weir (*Trametes*). A synonym of *Fomes tenuis*. (BPI-type.)

subflexibilis (Berk. & Curt.) Cooke (*Fomes*). Sessile, convex, up to 4 × 5 × 2 cm, glabrous; pores 8–9 per mm, the tubes papery; context reddish brown, with a thin crust, below soft and feltlike; setae none; spores hyaline or with a pale brown tint, oblong-ellipsoid, 3.5–4.5 × 2–2.5 μ. This fungus, although with a few tube layers, could be considered a species of *Polyporus*. Bresadola has annotated the type sheet "Le *Polyp. exotephrus* Berk. (1878) est la forme *annosa* de cette espèce, publié en 1868." Known from Cuba (K-type, BPI-isotype), and Costa Rica (BPI).

superficialis Schw. (*Boletus*). The Schweinitz material is sterile, but is almost certainly the same as *F. tenuis* as here recognized. (Phila. Acad. of Science, and BPI.)

taxodii Murr. (*Fomes*). Sessile, applanate to convex, glabrous; pores averaging 5 per mm; context reddish-brown, the hyphae 2.5–5 μ in diameter; setae subulate, 20–30 × 5–8 μ; spores absent on type, on other material ellipsoid, 4.5–5 × 2.5–3 μ. On cypress in Georgia and Florida; associated rot unknown. (Univ. Florida Herb.-type; NY; BPI.)

tenuis Karst. (*Fomes*). Resupinate and often widely effused to rarely sessile; pores 2–4 per mm; context yellowish- or reddish-brown, thin, homogeneous; setae narrowly subulate, 25–55(–80) × 5–11 μ; spores smooth, cylindrical, 5–9 × 1.5–2 μ. On wood of coniferous or deciduous trees throughout the United States and southern Canada; associated with a ring rot and with a white pocket rot. *Poria isabellina*, *Trametes setosus*, and very probably *Boletus superficialis* and *Polyporus viticola*, are synonyms.

texanus Murr. (*Pyropolyporus*). A synonym of *F. robustus*. (NY-type.)

torulosis (Pers.) Lloyd (*Fomes*). Specimens as reported by Lloyd (**1915**, p. 244) and Overholts (**1953**) appear to be perennial specimens of *Polyporus licnoides* Mont. (BPI.)

tsugina Murr. (*Fomitiporia*). A resupinate condition of *F. robustus* on wood of coniferous trees. (NY-type.)

viticola Schw. (*Polyporus*). Spores as found by Overholts have so far not been seen on the Schweinitz material. The fungus is almost

certainly same as *F. tenuis* as here recognized. (Phila. Acad. Science, and BPI.)

vorax Harkness (*Daedalea*). A synonym of *F. pini*. The type collection is apparently no longer in existence; a specimen communicated by Harkness is designated as the neotype. (FH.)

yamanoi Imazeki (*Cryptoderma*). This fungus, renamed in 1951, was described as a segregate from *F. pini* on the basis of a thinner pileus, paler context, more distinctly stratified tubes which are also shorter, tubes often stuffed with brighter hyphae, and spores turning brown more quickly. A collection by Weir in Idaho is cited in the original description. Dr. Imazeki has kindly sent a part of the Weir collection; the specimen does not appear to differ from *F. pini* as defined in this paper.

zealandicus Cooke (*Fomes*). (FIG. 1, d.) Sessile, usually applanate; pores averaging 5–7 per mm; context reddish-brown, the hyphae 2–3 μ in diameter; setae more or less ventricose, some to nearly all with curved apices, 24–41 × 6–11 μ; type sterile, spores in other material smooth, oval to subglobose, 4–5 × 2.5–3.5 μ. On the wood of deciduous trees from Tennessee (U. Tenn. Herb.; Pa. State College; BPI), South Carolina (BPI), Florida (Pa. State College), Puerto Rico (Pa. State College), Jamaica (NY), and Trinidad (NY); known also from Chile (BPI), East Tropical Africa (NY), the East Indies (BPI), and New Zealand (K-type and paratype; BPI-paratype); associated with a white pocket rot. *Pyropolyporus robinsoniae* is a synonym, and Overholts (**1953**, p. 86) uses the name *Fomes extensus* (Lév.) Cooke for this fungus.

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STUDIES IN THE GENUS CLATHROSPORA

L. E. Wehmeyer

(with 19 figures)

The genus *Clathrospora* was described briefly and figures of the type species *C. elynae* were given by Rabenhorst (**1857**). Later, he (**1869**) changed the name of the type species to *C. alpina*, because he found it to occur on other hosts than *Elyna*. This, however, was not legitimate and has not been recognized by later authors. He based the genus upon the clathrate and/or flattened spores which he later (**1869**) gave as having "episporio reticulato-costato" an appearance probably arising from the slight constrictions at the numerous transverse and vertical septa of the spore.

Some writers, as Winter (**1887**), have united the genus with *Pleospora*, whereas others, as Berlese (**1900**) and Saccardo (**1891**), have segregated all species with flattened ascospores in this genus. Müller (**1951**) has recently limited the genus more strictly to those species with numerous septa and a "costate" appearance, as in the type species.

As the above might suggest, there are three rather distinct groups of species with flattened, muriform ascospores. The present treatment is a broad one including all three of these groups in order to bring out their inter-relationships.

The first of these groups may well be looked upon as variations of certain established species of *Pleospora*. In species of this latter genus, when there is only one vertical wall inserted in a few, or even all, of the cells, these septa are usually arranged in a common plane or at a slight angle to such a common plane. This results in a spore which shows no vertical septa when viewed from the two "sides" and in this respect may simulate the flattened spores used by some authors to segregate the species of *Clathrospora*. This is particularly true when the spore is slightly thinner in one diameter (which is not always at right angles to the vertical septa), as it is in some species. These are confusing and intermediate species, and in this discussion the *Pleospora* binomials are retained for them, inasmuch as their retention in *Clathrospora* is somewhat doubtful.

The second group of species (Table I) is one deriving from *Clathro-*

TABLE I

No.	Host	Spores	Asci	Perithecia
482	Herbs	19.5–23×8.5–9×10–10.5	70–78×17–20	200–300
356	*Chlorogalum*	19.5–25×9–9.5×7–8	80–90×10–15	150–250
357	*Polygonum*	21–23(26)×9.5–10×7	75–90×17–19	200–350
358	*Syntheris*	21–31×10–12×7–9	75–95×21–24	200–300
361	*Sambucus*	21.5–26.5×9–11×9–10.5	85–89×21–22	200–400
434	*Prunus*	23–25×10–11×9–10		
359	*Lupinus*	23–26×10.5–11×9–10.5	88–98×19–21	200–250
431	*Valeriana*	23–26.5×12.5×8.5–9.5		
360	*Coleosanthus*	23–27×12.5–14×9–10		200–350
362	*Delphinium*	23–28×12.4×8–9	85–90×21–25	300–500
365	*Stephanomeria*	24–26×12.5×8–9	85–95×25–36	200–300
366	*Linum*	24–26.5×10–12.5×8.5–9		
367	*Brickelia*	24–27×11–12.5×9.5–10.5	70×26	250–300
368	*Pentstemon*	24–27×11–12.5×9.5–10	88–96×20	
363	*Coleosanthus*	24–28×14×10–11		
369	*Clematis*	24–28×10.5–12.5×7.5–9	78–95×19–23	350–400
364	*Eupatorium*	24–28.5×12.5–14×9–10	85–100×27–30	200–250
370	*Linaria*	25–27×10.5–12.5×9–10	70×23	
371	*Balsamorrhiza*	25–28.5×12.5×8.5–9.5	85–105×20–22	300
372	*Senecio*	25–29×11–12.5×8.5–9.5	78–97×23–26	
535	*Ligusticum*	25–30×11–12.5×8–10	90–100×31–35	250–400
373	*Silene*	25–32×14–15×9–10.5	100–125×26–32	200–250
374	*Hedysarum*	26–28×10–11×9	80–90×20–24	300–350
376	*Lupinus*	26–28×10–11×9		
378	*Linum*	26–28×13–14×9–11		250–350
761	Herbs	26–28.5×11.5–12.5×8.5–9	88–107×26	250–350
377	*Helianthella*	26–29×12.5×7.5–9	75–90×26–35	200–300
384	*Pentstemon*	26–30×12.5–13×9.5–10.5	95–120×21–25	350–400
383	*Balsamorrhiza*	26–35×15–17×10–13		
379	*Artemisia*	26.5–28×11–12.5×9–10.5	90–106×23–25	250–350
375	*Aquilegia*	26.5–28.5×11–14×9–10	80–90×20–24	300–350
380	*Carum*	26.5–30×12.5–14×9–10.5	88–115×23–25	300
381	*Cirsium*	26.5–30×12.5–14×9–10	70–90×18–23	300–400
382	*Cyanomarathrum*	26.5–32×9–12.5×9	88–106×21–25	150–300

PLEOSPORA DIPLOSPORA

385	*Sisyrinchium*	28×12.5–14×7–8.5		250–300
387	*Pentstemon*	28–30×14×11		
388	*Monardella*	28–30×13–14×11–12.5	80–95×26–30	300–350
386	*Helianthella*	28–30×15–16×11–12		200–250
422	*Sisyrinchium*	28–35×16–18×10–11	85–106×30	200–300
410	*Carpha*	30–35×16–17×9–10	95–100×26	200–300
409	*Asclepias*	30–35×14–19×8.5–10.5	60×48	100–200
411	*Monardella*	30–35×17–19×12.5	100–125×35	200–250
412	*Umbellifer*	(26)30–40×(14)16–21×(9) 11–14	125–160×26–30	250–350
437	*Castilleja*	32–34×16–18×9–11	125–135×26–28	250–350
435	*Anemone*	34–37.5×16–18×12.5–13	130–160×35	300–400
689	*Helianthus*	34–38×19–21×12–14	100–120×30–35	200–300
413	*Linum*	38–43×19–24×12.5–15	160–175×35–40	250–500
432	Soap plant	38–44×17–20×14–16	130–160×47–53	200–300

TABLE I—(*Continued*)

No.	Host	Spores	Asci	Perithecia
		PLEOSPORA PENTAMERA		
390	*Avena*	18–20×8.5–10.5×5.5–6	70×13–15	150–200
414b	Grass	19.5–23×10.5×6.5–7.5	85–90×17–18	200–250
391	Grass	20–25×9–10×5.5–6	70–90×17–20	200–250
392	*Matthiola*	21–5–23×9–10×7–8.5	78×18–20	150–200
393	*Agropyron*	22–26×10.5×7	70–80×19–22	300
395	*Ligusticum*	23–26×11–12×7–9	95–105×21–23	200–250
394	Herbs	23–26.5×10–12×7–9	88–105×21–25	250–300
397	*Thermopsis*	23–28×11–12.5		
396	*Delphinium*	23–28×11–13.5×7–9	85–90×21–25	200–250
702	*Alopecurus*	24–27.5×12–13×8.5–9	90–100×19–25	150
743	*Lasiogrostis*	25–29×10–12.5×8–8.5	70–125×18–24	150–200
399	*Lupinus*	26–30×12–14×8–9	88–97×23–26	200–250
402	*Lupinus*	26–32×11–13×8–10	85–90×18–21	250–300
415b	*Elymus*	26–34×12.5–15×8–10		200–300
401	*Elymus*	26–34×12.5–15×8–10	70–100×26	200–300
400	*Clematis*	26.5–30×11–12×7.5–8	95–115×21–23	250–350
403	Herbs	27–32×12–12.5×7–9		200–250
433b	*Elymus*	28–34×14–15×8–9		
		PLEOSPORA PLANISPORA		
414	Grass	24.5–26×12.5×7	85–90×17–18	200–250
415	*Elymus*	(26)28–41×13–17×8–10		150–200
416	*Pentstemon*	28–35×13–16×9–11	78–100×23–26	300
433	*Elymus*	28–41×12.5–16.5×8.5–10		250–350
425	*Elymus*	28.5–41×12.5–16×9–10	70–100×26	
430	*Cyanomarathrum*	32–35×13–16×9–10.5	106–125×17–21	

spora permunda (Cke.) Berl. These species have much more definitely flattened spores and a single vertical wall at right angles to the broad diameter, so that no vertical septa occur in the narrow or "edge" view. They also have a rather distinctive form and definitely belong in *Clathrospora* if the flattened spores are taken as the generic characteristic. They are found on both grasses and herbs and both monocotyledons and dicotyledons, but usually at higher altitudes or latitudes.

The third group (TABLE II) centers about the type species *C. elynae*. They again have definitely flattened spores but three or more rows of vertical septa, at right angles to the longer diameter and five or more transverse septa and have vertical septa in the end cells. They occur on the monocotyledonous families *Gramineae*, *Cyperaceae*, and *Juncaceae* and seem to show morphological differences correlated with their host plants.

The first six species discussed below belong to the first group, in which the ascospore form could be derived from species of *Pleospora* by a flat-

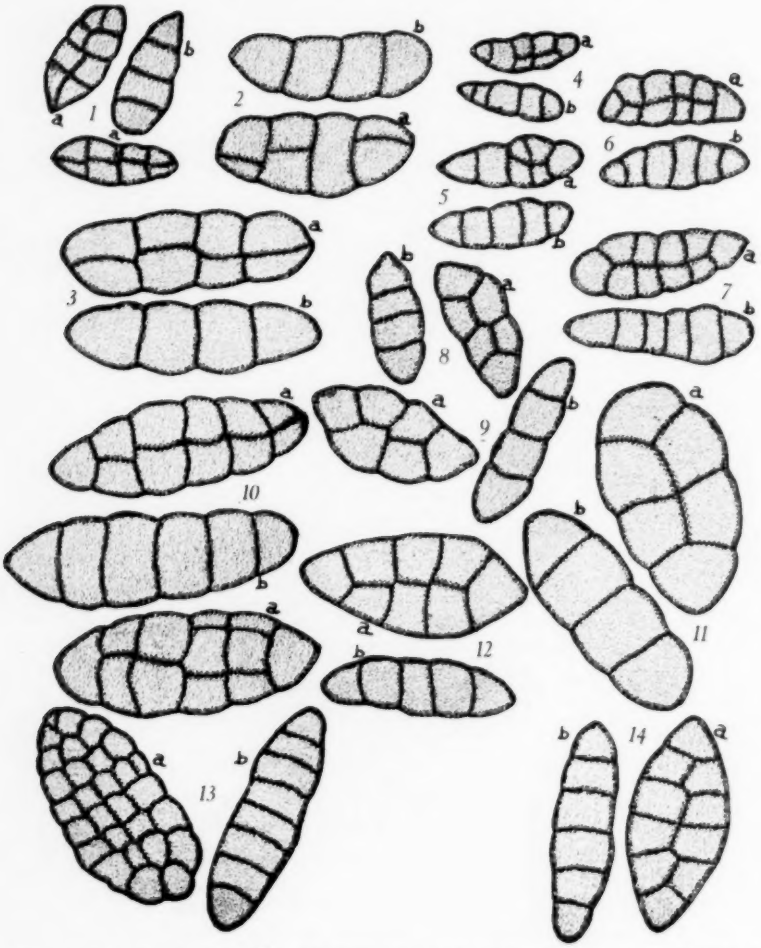
TABLE II

No.	Host	Spores	Asci	Perithecia
		CLATHROSPORA JUNCICOLA		
419	*Juncus*	32–35×13–18×9–12	88–145×26–28	200–300
		CLATHROSPORA TRISEPTATA		
557	*Juncus*	37–52×22–24×12–14	135–140×40–45	200–250
486	*Juncus*	49–59×23–26×12–14	130–160×45–53	200–250
		CLATHROSPORA MICROSPORA		
556	Sedge	32–36.5×13.5–15.5×9–10	75–90×30–38	100–150
		CLATHROSPORA SIMMONSII		
603a	*Juncus*	40–48×18–19×10–12	86–100×35–40	100–150
417	*Juncus*	46–59×26.5–28×9–11	100–130×40–45	200–300
487	*Juncus*	53–58×24–26×13–16	125–150×34–53	200–250
533	*Carex*	53–60×28–35×16–18	125–160×40–55	150–200
532	*Carex*	55–62×26–35×13–14	160×35	300–400
		CLATHROSPORA ELYNAE		
483	*Carex*	46–50×19–20×10–12	110–140×35–43	200–250
603	*Carex*	48–50×22–24×10–11	100–150×34–40	200
446	*Elyna*	48–53×19–21.5×9–12	120–140×33–36	200–250
485	*Carex*	50–55×21–24×10		200–250
484	*Carex*	53–56×21–25×10–12.5		200–300
		CLATHROSPORA BAKERI		
418	*Juncus*	53–58×20–24×11–12.5	140–175×35–43	200–300

tening of the spore. They are found, for the most part, on monocotyledonous hosts and many of them seem related to the *P. trichostoma* complex.

PLEOSPORA MALACOSPORA Speg. Mich. 1: 462. 1879. FIG. 1.

Perithecia 200–250 μ in diameter, globose, appearing as minute black dots; wall membranous, parenchymatous, smooth. Asci clavate, thick-walled, base somewhat claw-like, 75–80 × 16–18 μ. Spores biseriate, dark yellow-brown, 3-septate, cylindric- to oblong-ellipsoid, in face view, end rounded or finally broadly flattened, somewhat constricted at the septa, with one vertical septum in any or finally all cells; in edge view



FIGS. 1–14. Front view (a) and edge view (b) of ascospores of: 1. *Pleospora malacospora* Speg., from the type collection (No. *389*); 2. *Pleospora typhicola* (Cke.) Sacc., from collection No. *445*; 3. *Pleospora typhicola* (Cke.) Sacc., from the type collection (No. *762*); 4. *Pleospora ipomoeae* (Clem.) comb. nov., from the type collection (No. *404*); 5. *Pleospora ipomoeae* (Clem.) comb. nov., from collection No. *405*; 6. *Pleospora togwoticnsis* Wehm., from the type collection (No. *407*); 7. *Pleospora aquatica* Griffiths, from the type collection (No. *408*); 8. *Clathrospora permunda* (Cke.) Berl., from collection No. *356*; 9. *Clathrospora permunda* (Cke.) Berl., from collection No. *358*; 10. *Pleospora scirpicola* (DC.) Karst., from collection No. *727*; 11. *Clathrospora diplospora* (E. & E.) comb. nov., from collection No. *412*; 12. *Clathrospora pentamera* (Karst.) Berl., from collec-

cylindric-ellipsoid, straight or slightly inaequilateral, not constricted, and with no vertical septa, 17–21.5 × 8.5–9 × 6.5–7.5 μ.

Collections: No. *389* (Type) on old paper, from Italy.

The young spores are not strongly flattened and may lack vertical septa, but they become flattened, with squared ends and a continuous vertical septum at maturity. This species differs from the *Clathrospora permunda* series in the shape of the spores and the septation of the end cells, and from *Pleospora typhicola* in the darker brown and smaller spores.

PLEOSPORA TYPHICOLA (Cke.) Sacc. Syll. Fung. **2**: 264 (in Rel. Libert. 152). FIGS. 2, 3.

Sphaeria typhaecola Cke. Grev. **5**: 121. 1876.

Clathrospora typhicola (Cke.) Höhn. Ann. Myc. **16**: 89. 1918.

Clathrospora simulans Rehm, inedit. in Herb.

Pyrenophora typhicola (Cke.) Müll. Syd. **5**: 256. 1951.

Perithecia 300–350 μ in diameter, globose or somewhat depressed, scattered, immersed, erumpent as small papillate ostioles which appear upon the surface as tiny, scattered black dots; wall membranous, rather light yellow-brown, of small celled parenchyma and often with a fine tomentum of pale brown hyphae. Asci clavate, walls much thickened, particularly above, base claw-like, 135–180 × 26–34 μ. Spores biseriate, rather pale yellow-brown, 3-septate, somewhat flattened, oblong-ellipsoid, usually straight or slightly inequilateral, symmetric, more or less constricted at the septa, ends rounded, vertical septa in any or all of the cells including the end cells, similar in form in edge view, but without vertical walls, (28)35–53 × 11–16 × 9–13 μ.

Collections: *445, 746, 762* (Type), on *Typha angustifolia*, from England, Austria and Germany.

This is one of the questionable species of *Clathrospora*. The spores are only slightly flattened, if at all. There is only one vertical septum in each cell and they are in very nearly the same plane in all cells, so that one view of the spore shows no vertical septa. However, the vertical septa can be seen in views of the spore of varying diameter from 10–16 μ.

In Cooke's original description he cites "*Macrospora scirpi* Plow. Sph. Britt." In the Cooke Herbarium at Kew there are two collections labelled *Macrospora scirpi*, from North Ructon, one of May and the other of January, 1873. The May collection shows only a *Leptosphaeria*, but

tion No. *433*; 13. *Clathrospora juncicola* (E. & E.) comb. nov., from the type collection (No. *419*); 14. *Clathrospora planispora* (Ell.) Berl., from collection No. *416*.

the January collection, which was issued in Sphaer. Brit., II, No. *74* yielded one perithecium with immature spores, some of which were light brown, 47–53 × 10–14 μ and fit Cook's description. This is taken as the type specimen. A collection in the Höhnel Herbarium (*746*) shows the same spores but in greater abundance and excellent condition. These spores show the variability mentioned above. Höhnel (**1918**) gives the spores of his collection (*746*) as larger (48–65 × 17–23 × 13–14 μ); but measurements obtained by the writer were 43–51 × 13–15.5 × 10–14 μ. A collection in the Rehm Herbarium, in the Riksmuseum, labelled *Clathrospora simulans* Rehm, shows the same type of spore except that they are smaller (28–35 × 11–13 × 9–10.5 μ), somewhat more constricted at the septa and less bluntly rounded at the ends (FIG. 2). The similarity of the spore form, the perithecia and the host, however, indicate that it is the same as *P. typhicola*.

The characters of this species are similar to and overlap to some extent those of *Pleospora trichostoma*. The differences are these: *P. typhicola* has perithecia with a wall of lighter brown parenchyma which are slightly tomentose, not setose; the spores are smaller, narrower, are more strongly flattened and show more cells with vertical septa, which are often found in the end cells, which is never the case in *P. trichostoma*, *P.* (*Clathrospora*) *macrospora* (Schroet.) Nannf. differs only in the last character mentioned.

Pleospora Ipomoeae (Clem.) comb. nov. FIGS. 4, 5.

Comoclathris Ipomoeae Clem. Minn. Bot. Stud. **4**: 186. 1911.

Pyrenophora Ipomoeae Clem. In Crypt. Form. Colo. No. *450*.

Perithecia 150–300 μ in diameter, thickly scattered, immersed, globose to flattened-globose, sometimes collapsing, smooth or with a tomentum of rather stiff, brown hyphae; wall rather thin, parenchymatic. Asci clavate, with a thickened wall, short-stipitate with a claw-like base, 70–80 × 12–18 μ. Spores biseriate, yellow-brown, fusoid-ellipsoid to somewhat clavate, 3- or 4-septate, straight or slightly inaequilateral, often asymmetric, tapered below, constricted at the septa, somewhat flattened, one vertical septum in the second or second and third cell in face view, no vertical septa in edge view, cylindric to fusoid in edge view, 18–25 × 7–9.5 × 5.5–7 μ.

Collections: *404* (Type) and *405*, on *Ipomoea* and *Carex*, Colorado and Sweden.

This is a doubtful species and the two collections differ in certain respects. The spores are at first 3-septate, becoming 4-septate and they are only slightly flattened. In the type collection (*404*) the fourth

septum appears to be laid down in a leptosphaeroid manner in the lower central primary cell and vertical septa arise in the cells on either side of the original central septum. This spore is like that of *P. kansensis*, except that it is somewhat flattened and shows an edge view without vertical septa. The second collection (*405*) has spores in which a *vulgaris*-type septation occurs in the upper central cell of a 3-septate spore. Such spores appear like young spores of *P. vulgaris* except that they are also somewhat flattened. Further material is needed to show that such spores are constant and constitute a specific population, and are not merely immature spores of related species. Until that time these two collections are placed here under the binomial *P. ipomoeae*.

405 also has somewhat larger spores (21–25 × 8.5–9.5 × 5.5–7 μ) than *404* (17.5–25 × 7–8.5 × 5.5 μ) and its perithecia are more flattened and tomentose than those of No. *404*.

This species differs from *P. malacospora* and *P. typhicola* in the lack of vertical walls in the end cells and the additional fourth wall in many spores, and from *P. togwotiensis* and *P. aquatica* in the lack of the fifth septation in the spore.

PLEOSPORA TOGWOTIENSIS Wehm. Lloydia **9**: 231. 1946. FIG. 6.
Clathrospora tirolensis Rehm. Oest. bot. Zeitschr. **56**: 297. 1906. nomen nudum.

Perithecia 250–400 μ in diameter, depressed-globose, beneath the epidermis, smooth, thickly scattered, appearing upon the surface as minute circular blackened spots, with a minute central papillate ostiole; wall 20–30 μ thick, of coarse, dark brown parenchyma. Asci clavate, with a thickened apical wall and a claw-like base, 80–125 × 12–16 μ. Spores biseriate to overlapping uniseriate, yellow-brown to dark yellow-brown, fusoid-ellipsoid to ellipsoid, flattened, five-septate, contricted at the septa, inaequilateral to slightly curved, mostly symmetric, with one vertical septum passing through the four central cells but not the end cells, cylindric-ellipsoid, with no vertical septum in edge view, 19–28.5 × 7–11 × 5.5–8.5 μ.

Collections: *406*, *407* (Type), on grass and *Lupinus*, from Wyoming and the Tyrol.

This species was described from Wyoming by the writer, but the collection of Rehm (*406*) which he named *C. tirolensis* but did not describe is identical except that the spores are somewhat smaller (19–23 × 7–10 × 5.5–6.5 μ). The spores of this species are similar to those of *Pleospora vulgaris*, except that they are flattened and show no vertical septum in the narrow edge view.

PLEOSPORA AQUATICA Griffiths, Bull. Torr. Bot. Cl. **26**: 443. 1899. FIG. 7.

Perithecia somewhat flattened-globose, 100–250 × 100–150 μ, immersed, scattered and appearing as minute blackened dots, ostiole perforate almost invisible, causing a minute slit in the epidermis, wall 10–20 μ thick of coarse black parenchyma. Asci stout-clavate, thick-walled, with a claw-like base, 88–100 × 21–25 μ. Spores overlapping biseriate, pale yellow-brown, oblong- to fusoid-ellipsoid, flattened, 5-septate, mostly straight or slightly inaequilateral, usually somewhat asymmetric, more tapered below, constricted at the septa, with one vertical septum in several or all the central cells but none of the end cells, in face view; cylindric to cylindric-clavate, with no vertical walls in edge view, 26.5–33 × 10–12.5 × 9–10 μ.

Collection: *408* (Type), on *Heleocharis*, from South Dakota.

Griffiths states that the spores have 2–4 vertical septa and that they have a gelatinous envelope which extends at the ends as an appendage. His figures, however, show only one vertical septum in face view, and this is also true of the spores in the type collection. These spores do not show the gelatinous envelope, but this is often found only on fresh spores. These spores have the same form as those of *P. vulgaris* or *P. punctata* but are flattened in one plane.

Nannfeldt's collection (*794*) of *Pleospora macrospora* bears also a second species with flattened spores (*794a*) which are very similar to those of this species but are more symmetric, less tapered below, darker brown and often show an extra septum in the lower or also the upper end cell making them 6- or 7-septate. It may be distinct, but too little material has been seen to describe it as new.

PLEOSPORA SCIRPICOLA (DC.) Karst. Myc. Fenn. **2**: 72. 1871. FIG. 10.
Sphaeria scirpicola DC. Fl. Fr. **2**: 809. 1810.
Macrospora scirpi Fck. Symb. Myc. 139. 1870.
Macrospora scirpicola (DC.) Fck. Symb. Myc. Nachtr. **1**: 13. 1871.
Pyrenophora scirpicola (DC.) Muell. Sydowia **5**: 256. 1951.

On the surface as thickly scattered, slightly elongate, pustulate black dots, the erumpent ostioles of the globose perithecia, 250–350 μ diameter. Asci broad-clavate, very thick walled, base claw-like, 100–120 × 35–45 μ. Spores biseriate, ellipsoid, somewhat flattened, yellow-brown, somewhat constricted at the septa, ends rounded, with one or two vertical septa in any or all of the cells, coarsely guttulate, often slightly curved or inaequilateral, in edge view usually narrower, cylindric or inaequilateral and without vertical septa, 50–54 × 15–22 × 14–16 μ.

Collections: *727*, on *Scirpus*, from Germany.

This species is known to the writer only from Fuckel's exsiccatus (*727*). De Candolle's description of *Sphaeria scirpicola* is vague and without spore description and can scarcely be used to fix the species. Fries' exsiccatus of *Sphaeria scirpicola* DC. (Scler. Suec. *150*), as represented in the Farlow Herbarium, is a *Leptosphaeria* with three-septate spores and not the fungus issued by Fuckel. Fuckel's *Macrospora scirpicola* is, therefore, a misdetermination of Fries' fungus and the name belongs as a synonym of *Leptosphaeria*. The species epithet *scirpicola* is, however, still available in the genus *Pleospora* and is, therefore, retained here.

There is one view of the spores of this species which shows no vertical septa. The spore is usually, but not always, narrower in this view, but on the other hand vertical septa are occasionally found in the narrower or edge view of the spore. It is again one of the intermediate types of spore which would be placed in *Clathrospora* arbitrarily.

This species differs from *P. aquatica* chiefly in the larger spores.

The following four species, *Clathrospora permunda*, *C. diplospora*, *C. pentamera* and *C. planispora*, all have a similar and rather distinctive spore form, which is definitely flattened, for which reason these species are placed in *Clathrospora*. The species differ chiefly in the number of transverse septa in the spore. They are all distinct from the third or "elynae" group in the lesser number of septa, both transverse and vertical. They are the species most abundant in sub-alpine habitats and are found abundantly on herbaceous stems. The accompanying TABLE I will show the overlapping series of spore measurements which occur in such collections.

CLATHROSPORA PERMUNDA (Cke.) Berl. Nuov. Giorn. bot. ital. **20**: 195. 1888. FIG. 9.

Sphaeria permunda Cke. Grev. **5**: 111. 1876.
Pleospora compressa Harkn. Bull. Washb. Lab. Nat. Hist. **1**. 1884.
Pleospora permunda (Cke.) Sacc. Syll. Fung. **2**: 243. 1883.
Pleospora baccata Ell. Bull. Torr. Bot. Cl. **10**: 53. 1883.
Pleospora utahensis E. & E. Proc. Calif. Acad. Sci. **5**: *727*. 1895.
Pleospora silenes Earle, N. Y. Bot. Gard. Bull. **10**: 294. 1904.
Pleospora collomiae Rehm, in Herb.? (Riksmuseet).

Perithecia 150–250(–500)μ in diameter, usually thickly scattered, beneath the epidermis, flattened-spherical, collapsing pezizoid in age, with a radiating tomentum of rather stiff, coarse, dark brown hyphae which is visible beneath the epidermis, at least with a hand lens; wall

15–40 μ thick, composed of coarse, dark-walled parenchyma. Asci broad-clavate to cylindric-clavate, thick,walled, with a claw-like base, little or no interthecial tissue, 75–125 × 15–30(35) μ. Spores biseriate, yellow-brown to dark red-brown, fusoid, ellipsoid or somewhat clavate, flattened, cylindric to clavate in edge view, straight or often inaequilateral, symmetric or asymmetric with a broader upper and tapered lower portion, ends acutely tapered or broadly rounded, somewhat or not at all constricted at the septa, 3-septate, with vertical septa in the two central cells in face view, but no vertical septa in edge view, 20–30(–35) × 8.5–14(–17) × 7–10(–12) μ.

Collections: As listed in TABLE I. Type collection, *761*. On many hosts, chiefly herbaceous plants, from higher altitudes of western North America.

This is the most common and widespread of the species of *Clathrospora* and no doubt occurs in many sub-alpine or sub-arctic regions and has been described under many names. Cooke cites the type specimen as "on stems of soap plant, No. *244*." A Cooke specimen with this number (*244*) in the herbarium of Kew (*761*) is typical of the species here described. In the N. Y. Bot. Gard. there is a collection (*432*) from Harkness, on "soap plant," which might be taken as type material. This collection has larger spores (38–44.5 μ long), however, which are of the type placed in *C. diplospora* in this account.

There is a good deal of variation in size, form and color of the spores in this species and they grade off (in size) into the following species, *C. diplospora*. There is too much variation within collections, and too little correlation between collections, however, to recognize any acceptable varieties. Likewise it is necessary to erect an arbitrary separation from *C. diplospora* by placing all collections with some spores less than 28 μ in length in *C. permunda*.

The type material of *Pleospora baccata* Ell. (*381*), *P. silenes* Earle (*373*) and *P. utahensis* E. & E. are typical of *P. permunda* as described above. In his description of *P. utahensis*, Ellis states that the spores are finally about 7-septate. A few perithecia of *P. media* were found on his type material, and it was no doubt these spores which he saw. Ellis also states (1892) that the spores of *P. baccata* finally become 5-septate. This again must be due to the presence of a few spores of *P. vulgaris*, which were seen on the surface in the type material. The type (*357*) of *P. compressa* Harkn. has rather small, often clavate spores with refractive granules in each cell, which is sometimes seen in this species, but it falls within the species limits as here outlined.

It is, likewise, an open question as to whether the collections listed

under this and the following species, *C. diplospora,* should or should not be united under one species name. *C. permunda* is limited here, in a purely arbitrary fashion, to those collections which have at least some spores less than 28 μ in length. There are collections, as can be seen from TABLE I, which have a mixture of such smaller spores and also some larger ones as found in *C. diplospora.* Whether these are variable strains within a species group or hybrids between two species groups, there is at present no means of determining. Collection *412* is of interest in this connection. There are two types of perithecia intermixed upon this material. In one, all of the asci contain small spores, 23–26 × 11–12.5 μ, whereas in the other they all contain larger spores, 29–40 × 16–10 μ. Situations such as this suggest that there may be two or more strains or species which are able, in some way, to hybridize.

Clathrospora diplospora (E. & E.) comb. nov. FIG. 11.

Pleospora diplospora E. E. Erythea **2**: 20. 1894.

Clathrospora ellisiana Berl. Ic. Fung. **2**: 30. 1900.

Pleospora sisyrinchii Speg. Myc. Arg. ser. 2 (Anal. Mus. Nac. Nat. Buenos Aires ser. 3, **1**: 71). 1902.

Pleospora carphicola Speg. inedit. in Herb. La Plata Mus. No. *2148.*

Perithecia rather thickly scattered, immersed beneath the epidermis, flattened spheric, 100–350(500) μ in diameter, collapsing pezizoid in age, with more or less radiate, creeping dark brown tomentum about the base; wall 15–30 μ thick, of coarse dark parenchyma. Asci stout-clavate, wall somewhat thickened at the apex, base claw-like, 60–160 × 26–45(53) μ. Spores biseriate, yellow-brown to dark red-brown, ellipsoid, or somewhat clavate, mostly straight or inequilateral, symmetric or tapered below, ends mostly broadly rounded, sometimes acutely tapered, with or without constrictions at the septa, cylindric to clavate in edge view, 3-septate, with a single vertical septum in each of the central cells, but no vertical septa in edge view, 28–44 × 14–24 × (8)9–18 μ.

Collections: As listed in TABLE I, type collection *409,* on various herbaceous stems, from western North America and Patagonia.

As mentioned under *P. permunda,* this species is merely a continuation of the range of spore size, of that species. Collections with no spores less than 28 μ in length are arbitrarily placed in *P. diplospora* because this point seems to be the most distinct break in the series. Collections with spores at the two extremes of the spore range of these two species are quite distinct, but some others show a variation in spore size which overlaps the two species as here distinguished.

Ellis gives the spores of his *P. diplospora* as 3-, then 5- to 7-septate.

He shows drawings of such many-septate spores on his type packet and states that they occur in the same perithecia as the 3-septate ones. Such many-septate spores do occur in the type (*409*), but in separate perithecia, and Berlese's (**1900**) interpretation that this type is a mixture of *P. herbarum* and Ellis' species is the correct one. However, inasmuch as it is obvious that Ellis is describing a 3-septate, clathrate species, it does not seem necessary to change his name to *C. ellisiana,* as is done by Berlese.

The type (*385*) of *P. sisyrinchii* Speg. has spores which are mostly 28 μ in length. A second collection (*422*) and another marked *P. carphicola,* in the La Plata Museum, both from Patagonia, show the larger spores however, and they are, no doubt, all this species.

Collection *689* is determined as *Clathrospora macrospora* Schroet. This is actually an abnormal form or variety of *C. diplospora.* Some of the spores in this collection are typical of this species, whereas others are strongly tapered below, giving a clavate form not characteristic of this species. One or two spores were seen with four septa in edge view.

Petrak (**1940**) states that the type (Myc. Ven. *1361*) of *P. oligomera* Sacc. & Speg. (non Sacc.) is *P. permunda,* with spores 30–37 × 15–18 × 10 μ, which would be placed, according to our present conception, in *P. diplospora.* The copy of this exsiccatus in the Farl. Herb. shows only sterile or immature perithecia which yielded a few immature spores with one or two transverse septa. Petrak also states that *P. dissiliens* Magnus (spores 28–35(–39) × 15–18 × 10–12 μ), *P. sororia* Bub. (spores 30–41 × 15–18 × 10–15 μ), *P. curvasca* Bub. and *C. constricta* Maire (spores 29–36 × 16–19 × 11–15 μ) are synonyms.

CLATHROSPORA PENTAMERA (Karst.) Berl. Icones Fung. **2**: 31. 1900. FIG. 12.

Pleospora pentamera Karst., non Berl., Fung. Spitzb. p. 99. 1880.
Pleospora aurea Ellis, Bull. Torr. Bot. Cl. **10**: 53. 1883.
Pleospora quadriseptata Cke. & Harkn. Grev. **14**: 10. 1885.
Clathrospora quadriseptata (Cke. & Harkn.) Berl. Ic. Fung. **2**: 30. 1900.
Clathrospora aurea (Ell.) Berl. Ic. Fung. **2**: 31. 1900.
Pleospora turkestanica Rehm, Ann. Myc. **9**: 369. 1911.

Perithecia 150–300 μ in diameter, thickly scattered, flattened-spheric, collapsing in a pezizoid manner, with a radiate dark brown tomentum from their base, immersed beneath the epidermis; wall thin, 10–20 μ, parenchymatic. Asci clavate, thick-walled, with a claw-like base, 70–115 × 15–25 μ. Spores biseriate, fusoid-ellipsoid or rhomboid, 4-septate, not

constricted at the septa, ends bluntly tapered, symmetric or somewhat tapered below, straight, cylindric or somewhat tapered in edge-view, sometimes slightly curved, or slightly constricted at the septa; one vertical septum in the central cells in face view, none in edge view, (18–)20–34 × 8.5–15 × 6–10 μ.

Collections: as listed in TABLE II, on grasses and herbaceous stems, from California, Colorado, Nevada, Utah, Washington, Wyoming; Scandinavia, the Tyrol and Turkestan.

Another of the subalpine or subarctic species, differing from *P. permunda* and *P. diplospora* in the 4-septate spore. Type material of *Pleospora pentamera* Karst. has not been seen, but Berlese (**1900**, Pl. 46, fig. 3) shows 4-septate spores, typical of the material described here, from Karsten's original material. Karsten says the spores are rarely 5–6-septate, but there are often several species of *Clathrospora* on these stems. Collections *391* and *702* of *P. pentamera* from Europe both have this species present. *702* has a second *Pleospora*, probably *P. helecocharidis*, also present. As this name has priority, it is used here for this species. Berlese (**1900**, p. 31) says *C. aurea* differs from *C. pentamera* in the broader spores and collapsing tomentose perithecia, but these are variable characters.

There are two Harkness collections of *C. aurea* from the same locality, one made in June 1881 and the other in Feb. 1882, in the N. Bot. Garden. The earlier of these (*394*) is taken as the type, but both of them agree with the description as given above. Type material (*392*), in the California Academy Herbarium, of *P. quadriseptata* has a slightly smaller and clavate rather than rhomboid spores, but is no doubt this same species. The spores of the type (*743*) of *P. turkestanica* are mostly collapsed and in poor condition, but the normal ones are the same as *P. aurea*.

C. planispora, the following species, differs from this one only in the 5-septate instead of 4-septate spores. The two species often occur upon the same stem, which has caused some confusion in the literature.

According to Berlese's figures (**1900**, Pl. 46–47), *C. platyspora* (Sacc.) Berl. and *C. passeriniana* Berl. are this same species.

CLATHROSPORA PLANISPORA (Ell.) Berl. Nuov. Giorn. Bot. Ital. **20**: 200. 1888. FIG. 14.

Pleospora planispora Ell. Bull. Torr. Bot. Cl. **10**: 53. 1883.

Perithecia globose, 150–250 μ in diameter, thickly scattered beneath the epidermis as tiny black spots, with very little radiating tomentum, wall 10–15 μ thick, parenchymatous. Asci stout clavate, walls somewhat

thickened, base claw-like, 75–125 × 17–25 μ, imbedded in an interthecial tissue. Spores biseriate, broad fusoid-ellipsoid, yellow-brown to dark yellow-brown, 5-septate, mostly straight and symmetric, scarcely constricted at the septa, ends rounded or abruptly tapered, a single vertical septum in the four central cells, none in the end cells; in edge view cylindric, often inaequilateral or slightly curved, somewhat constricted at the septa, with no vertical septum (23)28–41 × 11–17 × 7–11 μ.

Collections: *414, 415, 416, 425, 430, 433* (Type), on stems of various herbs and grasses, Nevada, Utah, Wyoming, and the Tyrol.

There are three packets from the type collection (*433*) of *Pleospora planispora* in the N. Y. Bot. Gard. Herb., from which Ellis' N. Amer. Fung. *1584* was issued. Ellis' description and figures on the type packet give the spores as 4- or 5-septate, and a mixture of such spores are found in this material. A careful study of this material, however, shows that they are always in separate perithecia and that this collection is a mixture of *C. planispora* and *C. aurea*. In his published description, Ellis says that this species differs from his *P. aurea* in the 5-septate spores, so there is no doubt of his intended use of the binomial. Collection *414*, from the Tyrol, shows this same situation, for perithecia with somewhat smaller (19–23 × 10.5 × 6.5–7.5 μ) 4-septate spores can be found on the same section next to perithecia with somewhat larger (24.5–26 × 12.5 × 7 μ) 5-septate spores.

The remaining species are distinguished by the strongly flattened and also "clathrate" spores, which appearance is caused by the slight constriction at the numerous transverse (7–9) and vertical (3–7) septa, which give the spore a ridged appearance. They are found on members of the Cyperaceae and Juncaceae in semi-aquatic habitats. There seem to be morphologic differences associated with the host genera or even species which have not been distinguished in the past and more numerous collections are needed to bring out these interesting relationships.

Clathrospora juncicola (E. & E.) comb. nov. FIG. 13.

Pleospora juncicola E. & E. Proc. Phila. Acad. Sci. **1895**: 422.

Perithecia globose, 100–200 μ in diameter, immersed in the cortex, which tissue may be weathered away exposing the perithecia, thickly scattered as small black spots with a conic erumpent ostiole; wall thin, 5–10 μ, of coarse parenchyma. Asci broad-clavate, with a thickened wall and a claw-like base, 88–145 × 26–28 μ. Spores crowded, biseriate to triseriate, dark yellow-brown, ellipsoid, flattened, clathrate, straight or slightly inaequilateral, mostly symmetric, somewhat constricted at the

septa, with seven transverse and three vertical septa in face view; cylindric to cylindric-ellipsoid and with no vertical septa in edge view, 32–35 × 13–18 × 9–12 μ.

Collection: *419* (Type), on *Juncus balticus*, from Colorado.

Ellis' type collection of *Pleospora juncicola* shows the small spores with three vertical septa, here described. A second collection (*418*) has large spores with five vertical and often nine transverse septa and is described as a new species, *C. bakeri*.

Clathrospora triseptata sp. nov. FIG. 15.

Perithecia globosa, 200–250 μ diametro, dense dispersa, ostiolo papilliformi. Asci late clavati, crasse tunicati, basi unguiculiformes, 130–160 μ longi, 40–53 μ crassi. Sporae imbricate biseriatae, lutei-brunneae, ellipsoidales, 7-septatae, rectae, symetricae, ad septas leniter constrictae aspectu frontali verticaliter 3-septatae, aspectu laterali cylindraceae, deorsum attenuati, septis verticalibus invisis, 37–52 μ longae, 22–26 μ latae, 12–14 μ crassae.

Specimen typicum (n. *557*) vide infra enumerationem locorum, in caulibus *Junci drummondii*.

Perithecia globose, 200–250 μ in diameter, thickly scattered, appearing on the surface as numerous minute black spots, with papillate erumpent ostioles. Asci stout clavate, with a thickened wall and a claw-like base, 130–160 × 40–53 μ. Spores overlapping biseriate, yellow-brown, ellipsoid, 7-septate, mostly straight and symmetric, slightly constricted at the septa, ends rounded, with three vertical septa, in face view; cylindric or tapered below, with no vertical septa apparent in edge view, 37–52 × 22–26 × 12–14 μ.

Collections: *486, 557* (Type), on *Juncus trifidus* and *J. drummondii*, from Switzerland and California.

The two collections placed in this species differ from *C. juncicola* in the larger spores, and from all other species of this series in having only three vertical septa. *C. stipae* Trav. seems to be similar, according to its description, but has somewhat smaller spores which are given as having seven to nine transverse septa and differs in the host.

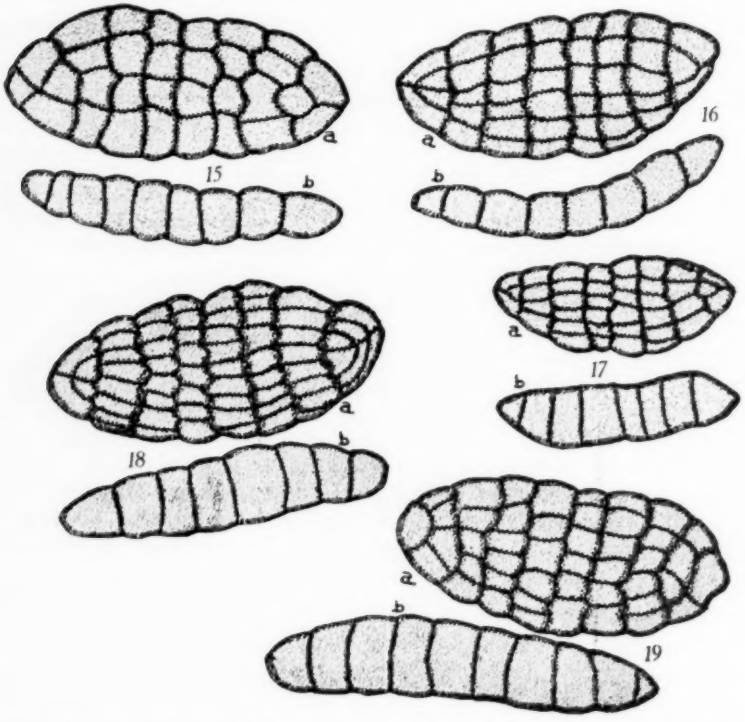
The two collections on the two different species of *Juncus* have spores which are slightly different in size and show a slight difference in the septation of the end cells, but this can be shown to be constant only by examination of further collections on these hosts.

Clathrospora microspora sp. nov. FIG. 17.

Perithecia globosa, 100–150 μ diametro, dense dispersa, superficialia ut maculae atrae, parvae, ostensa cum ostiolo centrali, vix erumpente. Asci late clavati, crasse tunicati, basi unguiculiformes, 75–90 μ longi, 30–38 μ crassi. Sporae bi- vel tri-

seriatae, ellipsoidales, clathratae, lutei-brunneae, in aspectu frontali 7-septatae, plerumque modice inequilaterales, ad septis paululo constrictae, deorsum attenuatae, verticaliter 5-septatae, in aspectu laterali cylindraceae, paululo curvatae, septis verticalibus invisis, 32–36.5 μ longae, 13.5–15.5 μ latae, 9–10 μ crassae.

Specimen typicum (n. *556*) vide infra enumerationem locorum, in caulibus Cyperacearum.



FIGS. 15–19. Front view (a) and edge view (b) of ascospores of: 15. *Clathrospora triseptata* sp. nov., from the type collection (No. *557*); 16. *Clathrospora simmonsii* Wehm., from collection No. *487*; 17. *Clathrospora microspora* sp. nov., from the type collection (No. *556*); 18. *Clathrospora elynae* Rab., from collection No. *603a*; 19. *Clathrospora bakeri* sp. nov., from the type collection (No. *418*).

Perithecia globose, 100–150 μ in diameter, appearing on the surface as minute, scattered, blackened dots; walls membranous. Asci stout clavate, with a thickened wall and a claw-like base, 75–90 × 30–38 μ. Spores biseriate to triseriate, fusoid-ellipsoid, flattened, clathrate, yellow-brown, 7-septate, usually somewhat inaequilateral, flattened on one edge, slightly asymmetric, more tapered below, slightly constricted at the

septa, with five vertical septa in the center of the spore in face view, cylindric to slightly curved and with no vertical septa apparent in edge view, 32–36.5 × 13.5–15.5 × 9–10 μ.

Collection: *556* (Type), on leaves of some sedge, from Greenland.

This species is based upon a collection in the Farlow Herbarium, made by H. E. Wetherill, on the Peary Auxilliary Expedition in 1894, at Cape York, Greenland. It appears to be upon leaves of some sedge. It has the septation of *C. simmonsii*, but has much smaller spores.

CLATHROSPORA SIMMONSII Wehm. Mycologia **44**: 652. 1952. FIG. 16.

Perithecia globose, 150–300 μ in diameter, immersed, appearing on the surface as thickly scattered small blackened spots with a central erumpent ostiole; wall 20–30 μ thick, outer layers of coarse black parenchyma. Asci stout clavate, with a thickened wall and a claw-like base, 90–160 × 35–55 μ. Spores overlapping biseriate, yellow-brown, ellipsoid to fusoid-ellipsoid, often inaequilateral, symmetric or slightly asymmetric, slightly constricted at the septa, clathrate, flattened, 7-septate or with an additional "Y" or "dome" shaped septum in the end cells, five vertical septa in the central cells of the face view; cylindric to clavate-cylindric and with no vertical septa in edge view, 40–62 × 18–35 × 9–14 μ.

Collections: *417, 487, 532* (Type), *533, 603a*, on *Carex ablata, Juncus jacquinii* and *J. mertensianus*, from the European Alps, California and Washington.

The collections cited on *Juncus* have spores slightly smaller than those originally described on *Carex*, but they are identical in form and septation. Fung. Eur. *2861* (*603, 603a*) contains two species of *Clathrospora* on two hosts; *603a* being the one on *Juncus jacquinii*.

The spores of this species differ from those of *C. elynae* in having only five, rather than seven, vertical septa, as in that species. The "Y" shaped or dome-like septa in the end cells are actually converging vertical septa and not transverse ones as in *C. bakeri* which has nine transverse septa.

CLATHROSPORA ELYNAE Rab. Hedw. **1**: 116. 1852. FIG. 18.

Pleospora elynae (Rab.) Ces. & de Not. Comm. Soc. Critt Ital. **1**: 218. 1861.

Clathrospora alpina Awd. Mycol. Eur. Pyren. **29**. 1869.

Perithecia 100–300 μ in diameter, globose, immersed, appearing on the surface as rather thickly scattered, tiny, round, black spots with a central papillate erumpent ostiole; wall 20–30 μ thick of coarse parenchyma,

blackened in the outer layers. Asci stout clavate with a much thickened wall and a claw-like base, 100–150 × 35–50 μ. Spores overlapping biseriate to triseriate, yellow-brown, ellipsoid, flattened, clathrate, usually inaequilateral, somewhat flattened on one side, and asymmetric, somewhat more strongly tapered toward one end, rounded at the ends, somewhat or not at all constricted at the septa, with seven transverse septa and seven vertical septa in the central rows, in face view; cylindric to cylindric fusoid, with no vertical septa in edge view, 40–56 × 18–25 × 9–12 μ.

Collections: *446, 483, 484, 485, 603,* on *Elyna spicata* and *Carex curvula* from southern Europe.

The figure given by Rabenhorst (**1857**) of the spore of *C. elynae* has seven transverse and seven vertical septa and all of the collections on *Elyna* and *Carex curvula* have spores of this type, and the specific epithet *elynae* should be applied to this group of collections.

This species differs from all of the preceding in the presence of seven vertical septa, in face view, in at least the two central rows of cells. There may be irregular, arching septa in the end cells which might be considered as transverse septa in addition to the seven always occurring in these spores, but these septa are actually converging vertical septa and not true transverse ones as those in the following species which is identical except for the nine distinct transverse septa. This species has been seen only on *Elyna spicata* and *Carex curvula.*

Pleospora heterospora de Not. is given as synonym of *C. elynae,* by Berlese (**1900**) and Mueller (**1951**, p. 259). The figure given by de Notaris (**1863**) of the spore has the shape of *P. herbarum,* but the numerous vertical septa of *C. elynae.* Berlese (**1900**) considers it a mixture of these two species.

Clathrospora bakeri sp. nov. FIG. 19.

Perithecia depresse globosa, 200–300 μ diametro, immersa, prominentia, dispersa; ostiolo papilliformi; muro 30–60 μ crasso, parenchymatoso. Asci late clavati, crasse membranacei, basi unguiculiformes, 146–175 μ longi, 35–43 μ crassi. Sporae imbricati-biseriatae, lutei-brunneae, in aspectu frontali fusiformes vel ellipsoidales, rectae, symmetricae, 9-septatae, ad septas paululo constrictae, verticali 5-septatae, in aspectu laterali cylindraceae, rectae, septis verticalibus invisis, 53–58 μ longae, 20–24 μ latae, 11–12.5 μ crassae.

Specimen typicum (n. *418*) vide infra enumerationem locorum, in caulibus *Junci,* in republica Coloradensi, leg. C. E. Baker.

Perithecia somewhat flattened-globose, 200–300 μ in diameter, immersed but rather prominent, scattered or in small clusters; ostiole papillate-erumpent; wall 30–60 μ thick of coarse black-brown paren-

chyma. Asci broad clavate, with a thickened apical wall and a claw-like base, 140–175 × 35–43 μ. Spores overlapping biseriate, yellow-brown, rather elongate-fusoid or ellipsoid, mostly straight and symmetric, slightly constricted at the septa, with nine transverse and five vertical septa in the center of the face view; long cylindric-fusoid and with no vertical septa apparent in edge view, 53–58 × 20–24 × 11–12.5 μ.

Collections: *418* (Type), on Juncus sp., from Colorado.

This collection is in the N. Y. Bot. Garden, under the name of *Pleospora juncicola,* but the spores are larger and differently septate than in that species. It differs from *C. simmonsii* in the two extra transverse septa. Whereas in *C. simmonsii* (FIG. 16) the end cells may show dome shaped septa with a single vertical septum, in *C. bakeri* (FIG. 19) the end cells contain two or more vertical septa, arising from a transverse septum which reaches to the lateral wall. This also results in the appearance of nine septa in the edge view of the spore.

CLATHROSPORA COOKEI Wehm. Mycologia **44**: 653. 1952.

This species has been described elsewhere. It has large (50–70 × 18–26 × 14–18 μ) spores with, mostly, 15 septa and an irregular type of septation. It is more or less intermediate between this genus and *Pleospora,* in that the spores are not strongly clathrate and that faint vertical septa can often be seen in the edge view of the spore.

Collections: *312* (Type), *530a,* on *Stipa californica* and *Muhlenbergia filiformis* from California and Washington.

In a recent paper, Mueller (**1951**) has described a new species, *Clathrospora multiseptata,* which, from the description and figure of the spore, appears to be the same or very similar to this species. Unfortunately Mueller cites a collection on *Phleum alpinum* under his Latin description and an entirely different collection, on *Draba aizoides,* under his German description, so that the type remains somewhat in doubt. If these two fungi prove to be the same, *C. Cookei* must become a synonym of *C. multiseptata.*

COLLECTIONS CITED

311 —*Pleospora pulchra* Kirschst., on *Typha latifolia,* Egelpfuhl, Kleinbehnitz, Kr. Werthavelland, Oct. 1, 1904, leg. W. Kirschstein. (Riksmuseet: Herb. Kirschstein) (Type).

312 —Undetermined, on *Stipa californica,* Horse Camp, Mt. Shasta, Calif., 8,000 feet, June 27, 1946, leg. W. B. Cooke *18095.* (Wehm. Herb.).

356 —*Pleospora permunda* Cke., on *Chlorogalum pomeridianum,* Sierra Nevada, Calif., leg. H. W. Harkness. (Riksmuseet: Rab. Fung. Eur. *3663*).

357 —***Pleospora compressa*** Harkn., on *Polygonum polymorphum*, Blue Canyon, Placer Co., Calif., June, 1882, leg. Harkness, No. *3284*. (Calif. Acad. Sci. Herb. No. *2833*) (Type).

358 —*Pleospora permunda* Cke., on *Syntheris dissecta*, Glory Mt., Jackson, Wyo., June 20, 1940. (Wehm. Herb. *1032*).

359 —*Pleospora permunda* Cke., on *Lupinus parviflorus*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1110c*).

360 —*Clathrospora permunda* (Cke.) Berlese, on *Coleosanthus umbellatus*, Big Canyon, Salt Lake Co., Utah, Aug. 24, 1906, leg. A. O. Garrett. (Riksmuseet: Fung. Col. *2417*).

361 —*Pleospora permunda* Cke., on *Sambucus microbotrys*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1133b*).

362 —*Pleospora permunda* Cke., on *Delphinium brownii*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1129a*).

363 —*Clasthrospora permunda* (Cke.) Berl., on *Coleosanthus grandiflorus*, Big Cottonwood Canyon, Salt Lake Co., Utah, Sept. 28, 1906, leg. A. O. Garrett (No. *965*). Riksmuseet: herb. Sydow).

364 —*Pleospora utahensis* E. & E., on *Eupatorium occidentale*, Utah, 1894, M. E. Jones. (N.Y.B.G.: Ellis coll. No. *5902*) (Type).

365 —*Pleospora permunda* Cke., on *Stephanomeria tenuifolia*, Red Hill Bridge, Gros Ventre River, Wyo., July 19, 1940. (Wehm. Herb. *1158*).

366 —*Pleospora permunda* Cke., on *Linum lewisii*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1134f*).

367 —*Pleospora permunda* Cke., on *Brickelia grandiflora*, Death Canyon, Teton Nat. Park, Wyo., Aug. 5, 1940. (Wehm. Herb. *1209*).

368 —*Pleospora permunda* Cke., on *Pentstemon stenosepalus*, Cream Puff Mt., Jackson, Wyo., July 5, 1940. (Wehm. Herb. *1086a*).

369 —*Pleospora permunda* Cke., on *Clematis douglassii*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1124b*).

370 —*Pleospora permunda* Cke., on *Linaria vulgaris*, Glory Mt., Jackson, Wyo., June 20, 1940. (Wehm. Herb. *1023b*).

371 —*Pleospora permunda* Cke., on *Balsamorrhiza sagittata*, Glory Mt., Jackson, Wyo., June 20, 1940. (Wehm. Herb. *1025*).

372 —*Pleospora permunda* Cke., on *Senecio* sp., Skyline Trail, Teton Nat. Park, Wyo., Aug. 5, 1940. (Wehm. Herb. *1208b*).

373 —*Pleospora silenes* Earle, on *Silene*, July 5, 1902, Carson, Nev., leg. C.F.B. No. *1241*. (N.Y.B.G.) (Type).

374 —*Pleospora permunda* Cke., on *Hedysarum uintahensis*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1126b*).

375 —*Clathrospora permunda* (Cke.) Berl., on *Aquilegia leptoceras*, Big Cottonwood Canyon, Salt Lake Co., Utah, Sept. 28, 1906, leg. A. O. Garrett. (Riksmuseet: Herb. Sydow).

376 —*Pleospora permunda* Cke., on *Lupinus parviflorus*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1130a*).

377 —*Pleospora permunda* Cke., on *Helianthella*, Togwotee Pass, Moran, Wyo., July 8, 1940. (Wehm. Herb. *1100a*).

378 —*Clathrospora permunda* (Cke.) Berl., on *Linum lewisii*, Big Cottonwood Canyon, Salt Lake Co., Utah, Sept. 10, 1906, leg. A. O. Garrett. (Riksmuseet: Herb. Sydow).

379 —*Pleospora permunda* Cke., on *Artemisia pedatifolia*. Togwotee Pass, Moran, Wyo., July 8, 1940. (Wehm. Herb. *1104*).

380 —*Pleospora permunda* Cke., on *Carum carui*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1132a*).

381 —*Pleospora baccata* Ell., on *Cirsium* sp., Utah, Territory, winter, 1882, leg. S. J. Harkness. (N.Y.B.G.: Ellis coll.) (Type).

382 —*Pleospora permunda* Cke. on *Cyanomarathrum parryi*, Glory Mt., Jackson, Wyo., June 20, 1940. (Wehm. Herb. *1028*).

383 —*Pleospora permunda* Cke., on *Balsamorrhiza sagittata*, Spencemont, Nev., 8300 feet, Oct. 31, 1892. (NYB coll. No. *8*).

384 —*Pleospora permunda* Cke., on *Penstemon* (?), Glory Mt., Jackson, Wyo., June 20, 1940. (Wehm. Herb. *1024*).

385 —*Pleospora sisyrinchii* Speg., on *Sisyrinchium junceum*, Rio Gallegos, Patagonia, 1900, leg. Hanthala. (La Plata Mus. No. *2155*) (Type).

386 —*Pleospora permunda* Cke., on *Helianthella multicaulis*, Spencemont, Nev., 8,300 feet, Oct. 31, 1892. (N.Y.B.G.: Ellis coll. No. *10*).

387 —*Pleospora permunda* Cke., on *Penstemon glaber*, var. *cyananthus*, Spencemont, Nev., 8,300 feet, Oct. 31, 1892. (N.Y.B.G.: Ellis coll. No. *9*).

388 —*Pleospora permunda* Cke., on *Monardella*, Kings Canyon, Ormsby Co., Nev., 1700–2000 m., July 1, 1902, leg. C. F. Baker. (Riksmuseet: Pl. Nev. No. *1213*).

389 —*Pleospora malacospora* Speg., on old paper, Italia: Conegliano, Feb. 23, 1879. (La Plata Mus. No. *7175*) (Type).

390 —Undetermined, on *Avena pratensis*, Gottland: Bro., June 20, 1920, leg. T. Vestergren. (Riksmuseet: Fung. Suec.).

391 —*Pleospora pentamera* Karst., on grass, beim Taschach, Gletscher in Pizthal (Tyrol), 6,000 feet, Dr. Rehm. (Riksmuseet: Rehm Herb.).

392 —*Pleospora quadriseptata* Cke. & Harkn., on *Matthiola*, Roseville, Placer Co., Calif., Feb. 1881. (Cal. Acad. Sci. Herb.: Harkness No. *1733, 2184*) (Type).

393 —*Pleospora aurea* Ell., on *Agropyron divergens*, Pullman, Wash., June 14, 1894, leg. C. V. Piper. (N.Y.B.G.: Wash. Pl. No. *265*).

394 —*Sphaeria* (*Pleospora*) *aurea* Ell., on herbs, Pleasant Valley, Utah, June 1881, leg. S. J. Harkness. (N.Y.B.G.: Ellis coll.) (Type).

395 —*Pleospora aurea* Ell., on *Ligustrum*, Pagosa Peak, Colo., 9,500 feet, Aug. 1899, leg. C. F. Baker. (N.Y.B.G.: Pl. S. Colo. *50*).

396 —*Pleospora aurea* Ell., on *Delphinium brownii*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1129b*).

399 —*Pleospora aurea* Ell., on *Lupinus parviflorus*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1110d*).

400 —*Pleospora aurea* Ell., on *Clematis douglassii*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1131b*).

402 —*Pleospora aurea* Ell., on *Lupinus parviflorus*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1130b*).

403 —*Pleospora aurea* Ell., on herbs, Pleasant Valley, Utah, Feb. 1882, S. J. Harkness. (N.Y.B.G.).

404 —*Pyrenophora ipomoeae* Clem., on *Ipomoea leptophylla*, Wray Co., Colo., 1,100 m., Aug. 25, 1907. (Farl. Herb.: Clem. Cr. Form. Colo. *450*) (Type).

405 —Undetermined, on *Carex arenaria*, Gottland: Vastkinde, s;n, Vastkinde, June 10, 1920, leg. T. Vestergren. (Riksmuseet: Fung. Suec.).

406 —*Clathrospora tirolensis* Rehm, on grass, bei der Konstanze Hutte (Tyrol), Sept. 1905, leg. Dr. Rehm. (Riksmuseet: Herb. Rehm) (Type).

407 —*Pleospora togwotiensis* Wehm., on *Lupinus candicans*, Togwotee Pass, Moran, Wyo., July 8, 1940. (Wehm. Herb. *1101d*).

408 —*Pleospora aquatica* Griff., on *Eleocharis palustris*, Aberdeen, South Dakota, May 1896, leg. D. Griffiths. (N.Y.B.G.: West. Am. Fung. No. *11*) (Type).

409 —*Pleospora diplospora* E. & E., on *Asclepias incarnata*, Rockport Co., Kansas, Feb. 7, 1893, leg. Bartholomew. (N.Y.B.G.: Ellis coll. No. *894*) (Type).

410 —*Pleospora carphicola* Speg. inedit., on *Carpha schoenoides*, Scholl Bay, Tierra del Fuego, Jan. 13, 1924. (La Plata Mus. No. *2148*) (Type).

411 —Undetermined, on *Monardella odoratissima*, Horse Camp, Mt. Shasta, California, 8,000 feet, June 27, 1856, leg. W. B. Cooke No. *18094*. (Wehm. Herb.).

412 —*Pleospora diplospora* E. & E., on *Umbellifer*, Glory Mt., Jackson, Wyo., 9,000 feet, June 20, 1940 (Wehm. Herb. *1026a*).

413 —*Pleospora diplospora* E. & E., on *Linum lewisii*, S. of Teton Pass, Jackson, Wyo., July 11, 1940. (Wehm. Herb. *1134a*).

414 —*Pleospora andropogonis* Niessl, on grass, Lilienthal, Tyrol, Sept. 1904, leg. Dr. Rehm. (Riksmuseet: Herb. Rehm) (5-septate component).

414b—Same data: four-septate *C. pentamera* component.

415 —*Pleospora planispora* Ell., on *Elymus*, Pleasant Valley, Utah, 7,000 feet, winter, 1882, S. J. Harkness. (N.Y.B.G.: Ell. N.A.F. *1584*) (2 pkts. 5-septate component).

415b—Same data: four-septate component.

416 —*Pleospora planispora* Ell., on *Pentstemon glaber*, Camp Davis, Jackson, Wyo., Aug. 3, 1940. (Wehm. Herb. *1201d*).

417 —*Pleospora elynae* Rab., on *Juncus mertensianus*, Horse Camp, Mt. Shasta, Calif., 8,000 feet, June 27, 1946, leg. W. C. Cooke No. *18427*. (Wehm. Herb.).

418 —*Pleospora juncicola* E. & E., on *Juncus* sp., Cameron Pass, Colo., 10,000 feet, July 15, 1896, leg. C. E. Baker. (N.Y.B.G.: Ellis coll. No. *429*).

419 —*Pleospora juncicola* E. & E., on *Juncus balticus*, North Park, Colo., 9,000 feet, July 22, 1894, leg. C. S. Crandall. (N.Y.B.G.: Ellis coll. *75*: Colo. Fung. *31*) (Type).

422 —*Pleospora sisyrhinchii* Speg., on *Sisyrhinchium middletoni*, Santa Cruz, Rio Galeggio, Jan. 1900, det. Linquist. (La Plata Mus. *13751*).

425 —*Pleospora planispora* Ell., on *Elymus condensata*, Spencemont, Nev., 8,300 feet, October 31, 1892. (N.Y.B.G. *12*) (5-septate component).

430 —*Pleospora planispora* Ell., on *Cyanomarathrum parryi*, Glory Mt., Jackson, Wyo., June 20, 1940. (Wehm. Herb. *1028b*).

431 —*Pleospora permunda* Cke., on *Valeriana*, Jackson, Wyo., July 1940. (Wehm. Herb. *1128*).

432 —*Pleospora permunda* Cke., on Soap plant, Blue Canyon, Calif., leg. Dr. Harkness. (N.Y.B.G.: Ellis coll. *3525*) (Type).

433 —*Pleospora planispora* Ell., on grass (*Elymus*), Pleasant Valley, Utah, 7,000 feet, winter, 1882, leg. S. J. Harkness. (N.Y.B.G.: Ellis coll. *415*) (Type).

433b—Same data: 4-septate component.

434 —*Clathrospora collomiae* Rehm, on *Prunus cerasus*, Yates, N. Y., April 11, 1906, leg., C. E. Fairman No. *23*. (Riksmuseet: Rehm Herb.).

435 —*Clathrospora diplospora* E. & E., on *Anemone occidentalis*, Panther Creek Meadow, Mt. Shasta, Calif., July 7, 1947, leg. W. B. Cooke *20285*. (Wehm. Herb.).

437 —*Clathrospora diplospora* E. & E., on *Castilleja miniata*, Panther Creek Meadow, Mt. Shasta, Calif., July 10, 1947, leg. W. B. Cooke. (Wehm. Herb.).

445 —*Clathrospora simulans* Rehm inedit., on *Typha* (*angustifolia?*), bei Schandau, April 1902, W. Krieger. (Riksmuseet: Rehm Herb.) (Type).

446 —*Pleospora elynae* Rab., on *Elyna spicata*, Wallis bei Zermatz, Sept. 1886, leg. T. Magnus. (Riksmuseet: Herb. Rehm).

482 —*Sphaeria permunda* Cke., on herbs, Pleasant Valley, Utah, S. J. Harkness. (N.Y.B.G.: N.A.F. *886*).

483 —*Pleospora elynae* Rab., on *Carex curvula*, Albula Pass, Echweiz, leg. com. Pazschke. (Riksmuseet: Rehm Asc. *1590*).

484 —*Pleospora elynae* Rab., on *Carex curvula*, Schweiz, Furkenpasshohe, 2,450 m., Sept. 1905, leg. O. Jaap. (Riksmuseet: Jaap Fung. sel. exs. *156*).

485 —*Pleospora elynae* Rab., on *Carex curvula*, Riva-Valdobbia (Novara), Sept. 1898, leg. A. Carestia. (Riksmuseet: Sacc. Myc. ital. *489*).

486 —*Pleospora elynae* Rab., on *Juncus trifidus*, Schweiz, Simplonhospiz, 2,000 m., July 29, 1905, leg. O. Jaap. (Riksmuseet: Herb. Rehm).

487 —*Pleospora elynae* Rab., on *Juncus jacquini*, Schweiz: Furkpasshohe, 2,430 m., Sept. 3, 1905. (Riksmuseet No. *305*).

531 —*Pleospora asymmetrica* Wehm., on *Lupinus volcanicus*, Dege Peak Trail, Mt. Rainier, Wash., 6,800 feet, Aug. 19, 1948, leg. E. G. Simmons No. *1937b*.

532 —*Clathrosporia simmonsii* Wehm., on *Carex ablata*, Burrough's Mt., Mt. Rainier, 7,400 feet, August 20, 1948, leg. E. G. Simmons No. *1930*.

535 —*Clathrospora permunda* (Cke.) Berl., on *Ligusticum purpureum*, Yakima Park, Mt. Rainier, Wash., 6,400 feet, Aug. 18, 1948, leg. E. G. Simmons No. *1952a*.

556 —*Clathrospora alpina* Awd., Cape York, N. W. Greenland, leg. H. E. Wetherill, July 23, 1894. (Farl. Herb., ex-Peary Aux. Exp.).

557 —*Clathrospora elynae* Rab., on *Juncus drummondii*, Glen Pass, Fresno Co., Calif., leg. J. Clemens, July 22, 1910. (Farl. Herb.).

603 —*Clathrospora alpina* Awd., on *Carex curvula* (and *Juncus jaquinii*), Rhetiae, July 1882, leg. H. Wegelin. (Farl. Herb. & Riksmuseet, Rab. Fung. Eur. *2861*).

603a—*Clathrospora alpina* Awd., on *Juncus jaquinii* (same exs.).

689 —*Pleospora macrospora* Schroet., on *Helianthella californica*, Jonesville, Butte Co., June 24, 1941, leg. E. B. Copeland, det. Lee Bonar. (Farl. Herb., Fung. Calif.).

702 —*Clathrospora pentamera* (Karst.) Berl., on *Alopecurus alpinus*, Bellsound, July 1938, leg. J. Vahl, det. J. Lind. (Farl. Herb., Mus. bot. Haun., Pl. Arctica).

727 —*Sphaeria scirpi* Fr., on *Scirpus lacustris*, hieme, Hattenheim. (Farl. Herb., Fck. Fung. Rhen. *859*).

743 —*Pleospora turkestanica* Rehm, on *Lasiogrostis splendens*, Tschelkar, prov. Turgaj (Turkestan), Oct. 1910, leg. Androssow. (Farl. Herb. & Riksmuseet, Tranzsch. & Ser. Myc. Ros. *123*) (Type).

746 —*Clathrospora typhicola* (Cke.) Höhn., on *Typha angustifolia*, Sonntagsburg, 1906, leg. G. Staasser. (Farl. Herb., ex Höhn. Herb. *A 3725*).

761 —*Sphaeria permunda*, S. Francisco, 1876, No. *244*. (Kew Herb., ex Herb. M. C. Cooke) (Type).

762 —*Macrospora scirpi* Fck., on *Typha latifolia*, North Ructon, May 1, 1873. (Kew Herb., ex Herb. M. C. Cooke) (Type).

794 —*Pleospora macrospora* Schroet., on *Hierochloe alpina*, Torne, Lappmark, Jukkasjarvi, s:n., Nissontjärro, July *23*, 1923, leg. J. A. Nannfeldt. (Fl. suecica *446*).

794a—Same data; second fungus.

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PERMANENT UNSTAINED MOUNTS OF FUNGI [1]

ALEXANDER BAKERSPIGEL [2]

(WITH 1 FIGURE)

In spite of certain methods described in recent years (**1**, **2**, **3**, **4**, **5**) it is still difficult to prepare permanent stained mounts of fungi in tissues and in culture. Since mounts of epidermal and other tissues from mycotic lesions are not sections, but thicker scrapings and exfoliations, it is necessary that the maximum transparency be preserved. In fact staining skin or nail scrapings infected with a dermatophyte often obscures rather than reveals the morphology and cytoplasmic contents of host and fungus cells.

Hairs infected with *Microsporum* are surrounded by a sheath of spores. When such hairs are passed through many solutions during staining, the spores may become lost. Similarly in cultures air-borne spores of such fungi as the dermatophyte *Trichophyton* or the common contaminant *Aspergillus* may also become loosened when subjected to many solution changes.

The method to be described here is used for the routine preparation of permanent mounts in this laboratory. It eliminates staining and substitutes air-drying at room temperature for liquid dehydrants, thus avoiding a number of solutions commonly used during staining, dehydrating and clearing, and produces satisfactory permanent unstained mounts of fungi.

METHOD

Fungi in Tissues: Infected hairs, and skin and nail scrapings are placed on a clean glass slide and cleared in a few drops of 10% KOH for 3–5 minutes. Thicker pieces of skin or nail scrapings may be cleared for longer periods.

[1] Presented before the Mycological Society of America Meeting held in conjunction with the American Institute of Biological Sciences at the University of Wisconsin, Madison, Wisconsin, September 9, 1953.

[2] Contribution from the Provincial Laboratory of Public Health, University of Alberta. This work was made possible by a Provincial Health Grant to Dr. E. S. Dowding.

After clearing, the specimen is washed with distilled water for 1–3 minutes. Washing may be prolonged depending on the thickness of the tissue and the type of infection.

Most of the water is then drawn off with fine blotting or lens paper and the specimen is left to dry in air at room temperature. The tissue dries slowly, shows a minimum of distortion and finally becomes fixed to the slide.

A permanent mount is made by pipetting a few drops of synthetic resin (Piccolyte or Harleco Synthetic Resin) [3] over the specimen and then applying a cover-slip.

Fungi in Culture: A Van Tiegham cell-culture of the fungus is made and when spores or other desired structures are obtained, the cover slip containing the culture is removed and dried in air. Cultures of pathogenic fungi such as *Phialophora verrucosa* can be killed with ether fumes before drying. After the culture has dried a permanent mount is made by inverting the cover slip and applying it to synthetic resin on a glass slide.

Stained Mycelium: It is surprising how little distortion there is when small mats of stained mycelium are air-dried before mounting. In fact this method is used here in cytological work and has produced very satisfactory permanent mounts of mycelium stained to show nuclei. Permanent mounts of the dried mycelium are made in a similar manner to that described for mounting fungi in tissues.

RESULTS

FIGURE 1, a–f, illustrates how satisfactory permanent unstained mounts of fungi in tissues may be when prepared by the above method. They show: a, *Trichophyton rubrum* in thumb nail; b, *Candida albicans* in inframammary skin; c, *T. mentagrophytes* in beard-hair; d, Mosaic fungus in skin from foot; e, *T. rubrum* in skin from foot, and f, *T. mentagrophytes* in skin from arm.

FIGURE 1, g, is a portion of a Van Tiegham cell-culture of *Phialophora verrucosa* showing in the right upper part spore-masses at the mouths of the cup-shaped phialides. All of these mounts are 18 months old.

This method is unsuitable for making permanent mounts of such fungi as *Neurospora* because of the distortion that occurs when the large thin-walled cells are air-dried. In addition it must be emphasized that mounts cannot be mass-produced and that each specimen should be done indi-

[3] Harleco Synthetic Resin—Hartman-Leddon Co., Philadelphia, Pa.; Piccolyte—General Biological Supply House Inc. (Turtox), Chicago, Illinois.

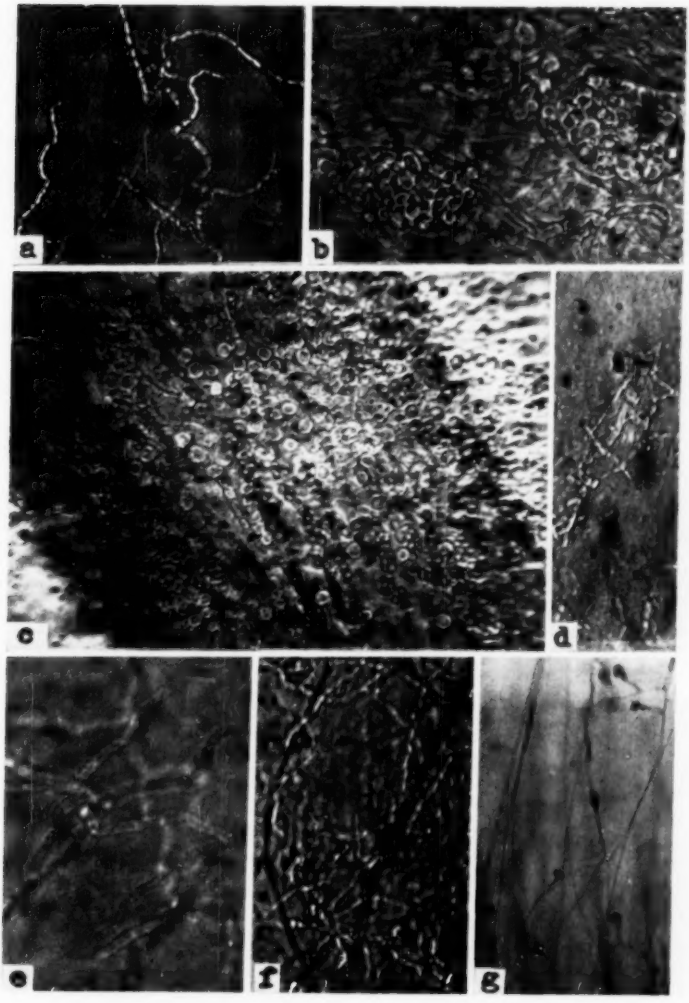

FIG. 1. Photomicrographs of 18 months old unstained mounts of fungi in tissues and in culture. a. *Trichophyton rubrum* in thumbnail; b. *Candida albicans* in inframammary skin; c. *Trichophyton mentagrophytes* in beard-hair; d. Mosaic fungus in skin from foot; e. *Trichophyton rubrum* in skin from foot; f. *Trichophyton mentagrophytes* in skin from arm; g. Van Tiegham cell-culture of *Phialophora verrucosa* showing, in the upper right, spore-masses at mouths of the cup-shaped phialides. Magnification of a, c, d, f and g × 334; b and e × 776.

vidually. Nevertheless, unstained mounts of many fungi in tissues and in culture can be made and remain unchanged even after 18 months.

CONCLUSION

Permanent unstained mounts of fungi in tissues and in culture can be prepared by eliminating a number of solutions commonly used during staining, and by substituting air-drying at room temperature for liquid dehydrants. Such mounts are useful for teaching purposes and other studies since they reveal the morphology of, and natural relationship between, host and fungus.

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NOTES AND BRIEF ARTICLES

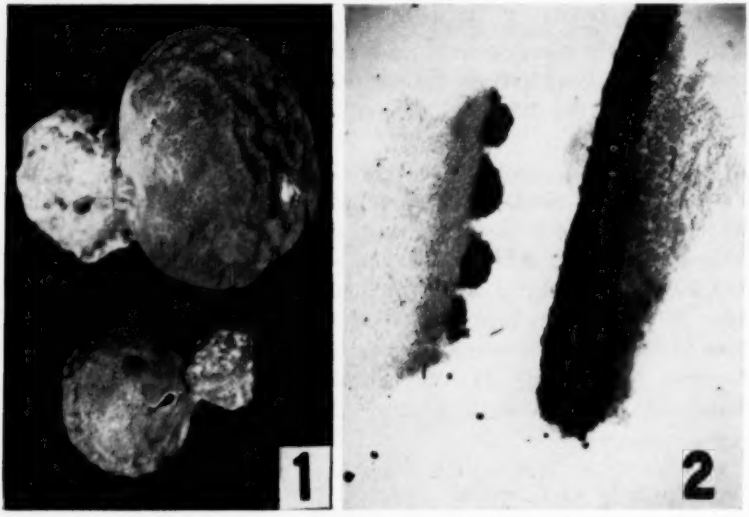
A new species of Scleroderma from Panama

On August 10, 1937, I found on Barro Colorado Island in the Panama Canal Zone the empty peridium of a *Scleroderma*, noteworthy by reason of its deep subglobose cup occupying a pocket in the hard soil, its regular stellate marginal lobes and particularly its brilliant golden-yellow interior. I sent it to the late Dr. W. C. Coker, who wrote me that it was unknown to him. I failed to find it when visiting the Island in 1945, but in 1952 Mr. A. L. Welden and I gathered several collections. Search of the literature reveals references to several Sclerodermas with a yellow or golden peridium, but the descriptions of none of them apply satisfactorily to the Barro Colorado collections. *S. endoxanthum* Petch (Ann. R. Bot. Gard. Peradeniya **7**: 76. 1919) is much larger, up to 10 cm in diameter unopened, depressed-globose and vertically sulcate, as opposed to the much smaller size, not exceeding 3 cm. unopened, and the regular globose form of the Panama species. Petch did not observe dehiscence, whereas dehiscence in the Panama species appears to occur very soon after maturity, so that it is hard to find an indehiscent specimen. Petch suggested that *S. vulgare* var. *novoguicense* Hennings was close to the Ceylon species. The description of the latter as given in Saccardo (Syll. Fung. **11**: 167. 1895) reads in part: "irregulariter dehiscente, substipitatum v. ramosoradicatum," which could not apply to the Panama species.

Another *Scleroderma* with a yellow inner peridium is *S. luteum* Lloyd (Myc. Writ. **4**, Letter **51**: 2. 1914), from Java. Through the courtesy of Mr. John A. Stevenson, I have been able to examine the type. Dr. Donald P. Rogers, of the New York Botanical Garden, has let me examine part of a collection by H. E. Parks, from Fiji, referred to the same species, correctly, I believe. Neither collection was dehiscent, but in size and aspect they are similar to the Panama collections, from which they differ, however, in several respects. In addition to the lack of evidence of early dehiscence, they are distinguishable in other ways, as shown in the following table based on two indehiscent basidiocarps of comparable size and stage of development.

	S. luteum (Fiji)	*S.* sp. (Panama)
Peridium	Smooth, 2 mm thick when dry	Warted, 1 mm thick when dry
Gleba	Firmly agglutinated	Very friable, falling away readily
Capillitium	None	Persistent
Spores	Body 5.5–6, over-all 6.5–8μ, dark yellow-brown, strongly spiny-reticulate; halo slight	Body 6–7.5, over-all 6.5–8μ, yellow-brown, indistinctly reticulate; spines much shorter, halo prominent

As Lloyd's species has not been illustrated, I reproduce a photograph of the type (FIG. 1). I also show an enlargement of sections through the peridia of the Fiji collection of *S. luteum* and through that of the Panama form, showing the smooth peridium of the former and the warted peridium of the latter (FIG. 2).



FIGS. 1, 2. 1. *Scleroderma luteum* Lloyd, Type, × 1. 2. Sections through peridium of *S. chrysastrum*, left, and *S. luteum*, right, × 22.

On the basis of these differences, and with particular emphasis on the deeply buried base, the early dehiscence and almost immediate dispersal of the spores and capillitium and the brilliant golden, star-like appearance of the empty peridium, I venture to describe this as new.

Scleroderma chrysastrum sp. nov.

Globosum, atrobrunneum, subhypogeum, solitarium vel saepius gregarium, usque 3 cm diam.; peridio duro usque 1 mm crasso, extus nigrobrunneo, superficie minute

squamuloso, intus aureo-flavo, in 9–12 segmenta regularia dehiscente; gleba nigricante compacta, celeriter evanescente; sporis globosis, purpureobrunneis, acute spinulosis et superficie subreticulatis, 6.5–8 μ diam.[1]

Globose or depressed-globose, dark brown, subhypogeic, solitary or more often gregarious, up to 3 cm in diameter before dehiscence; peridium hard, brittle, up to 1 mm thick, the dark exterior covered with blackish squamules to about the middle, internally brilliant golden yellow, splitting at dehiscence into 9–12 regular, triangular, spreading segments; gleba purplish black, compact, rapidly disappearing; capillitium scanty, of hyaline, often irregular hyphae; spores globose, with spines partly arranged in lines and forming a partial reticulation, 6.5–8 μ in diameter, including the spines.

Panama Canal Zone: Barro Colorado Island, 2 July 1952, G. W. Martin and A. L. Welden *7367* (type); 7 July 1952, G. W. M. and A. L. W. *7472;* 13 July 1952, G. W. M. and A. L. W. *7696.*[2]

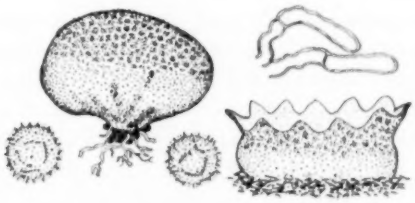


FIG. 3. *Scleroderma chrysastrum,* Habit, closed and open, × 1; spores and capillitium, × 1000.

The fruiting season appears to be brief. All good collections were made during the first half of July. On August 24, an old, dull, empty peridium was collected, but in 1952 the species appeared, then disappeared, within two weeks, although the same areas were collected intensively from late June to late August.—G. W. MARTIN, STATE UNIVERSITY OF IOWA, IOWA CITY.

SYNCHYTRIUM MODIOLIENSIS COOK AND SYNCHYTRIUM AUSTRALE SPEGAZZINI

During the period from 1945 to 1953 Cook created a large number of new species of *Synchytrium* which he collected in the vicinity of Baton Rouge, Louisiana. Most of these species were studied from the standpoint of gall structure which he believed to be more specific and diagnostic than the characteristics of the fungi in question. As a con-

[1] I am indebted to Dr. Rogers for correcting the Latin diagnosis.

[2] Collections made under National Science Foundation Grant G37.

sequence, Cook neglected the fungi to a large extent and did not contribute very much to our knowledge of their developmental phases, life cycles and morphology. Also, several of the species appear to have been established on the basis of their occurrence on one host in nature, and seldom were they carefully compared with earlier-reported and better known species which occur on the same hosts.

A good example of the point in question is *S. modioliensis* which Cook [1] created for a parasite on *Modiola caroliniana*. Apparently, this species was collected earlier from the same locality by Langlois (**1888**) and in Alabama by Carver (**1897**) on *M. multifida*, which is synonymous with *M. caroliniana*. Jennings (**1890**) and Bartholomew (**1902**) also collected what may possibly be the same parasite on *Callirrhoë involucrata* in Texas and Kansas, respectively. The herbarium specimens of Langlois, Carver, Jennings and Bartholomew at the New York Botanical Garden and elsewhere, however, bear the name *S. australe* Spegazzini. In a letter to Langlois which is filed with the specimens in New York and appears to be an acknowledgment of receipt of Langlois herbarium material, W. G. Farlow referred to the species as *S. langloisii* but added that he believed it to be *S. australe*. Probably for this reason and its occurrence on a differently named host, *M. multifida*, Cook regarded the fungus collected by Langlois and the others mentioned as distinct and different from his *S. modioliensis*. However, a careful study of Langlois' specimens by the author indicated that they might be the same species. The question then arose of whether or not Cook's species is identical with the one Spegazzini [2] collected in 1880 on *M. prostrata* in Argentina and gave the name *S. australe*. Spegazzini's diagnosis was very brief, without illustrations, and he described only resting spores which were 25–30 × 25 μ in diameter, with a thick hyaline epispore and coarsely granular content. Cook's diagnosis likewise was incomplete and included only a description of the sporangial galls (18 × 24 μ diam.), sori (14 × 18 μ diam.) and sporangia ($3\frac{1}{2}$ × 7 μ diam.).

From material collected at Baton Rouge, La., in March 1953 and grown on *M. caroliniana* and other malvaceous hosts in the greenhouse at Purdue the author has made an intensive study of host range, host reaction, gall formation, development and life cycle of *S. modioliensis*, and become familiar with its variations. Recently, through the kindness of Dr. J. C. Lindquist of the Instituto de Botanico Spegazzini, La Ciudad Eva Peron, Argentina, the author received type specimens of Spegaz-

[1] Cook, M. T. Species of *Synchytrium* in Louisiana. I. Mycologia **37**: 284–294.

[2] Spegazzini, C. 1881. Fungi Argentini, pt. 4. Ann. Soc. Cient. Argentina **11**: 1–138.

zini's *S. australe* and has had the opportunity of comparing it with *S. modioliensis*. These specimens were very old and fragile but contained numerous galls which could be studied. In addition to examination of the dried material, small, heavily infected leaf fragments were thoroughly soaked in water, placed in a vaccum to remove the included air, fixed, dehydrated, embedded in paraffin, sectioned at 7 μ, and stained. Even after being dried since 1880, the leaf tissues and galls were fairly well preserved, and in a few instances the nuclei of the sheath cells and sporangia were clearly recognizable.

These leaf sections included both sporangial and resting spore galls. The former are fairly large, 72–90 × 80–126 μ, protruding, composite and multicellular, and usually asymmetrical in relation to the subapical opening. The sporangial sorus is surrounded by a two-celled layer or sheath of cells which were badly shrunken and collapsed. In the dried material these galls have a dark lavender-red or dubonnet color. In a few of the sectioned and stained galls, what appear to be the remnants of a prosoral membrane are present, and the sporangial sori vary from 45 to 70 μ in greatest diameter. The sporangia are well preserved and vary from oblong, 13–19 × 18–33 μ, oval, 18–32 × 21–37 μ, subspherical, 24–32 μ, polyhedral, elongate and irregular in shape. In the dried material they are yellowish-orange in color with finely granular content and a thin hyaline wall. The resting spore galls are very abundant on both surfaces of the leaf and vary from oval, 50–54 × 60–72 μ, oblong, 38–42 × 58–69 μ, subspherical, 30–48 μ, elongate, and somewhat hemispherical. The lower portion usually extends down into the palisade or mesophyll parenchyma, while the upper part protrudes slightly or markedly above the surface of the leaf. They are predominantly unicellular and simple and usually consist only of an enlarged epidermal cell. Unfortunately, most of the resting spores had been torn out of the galls during sectioning and very few were available for measurement. These vary from oval to subspherical, 22–46 μ, in shape with a thick wall and coarsely granular content. Spegazzini described the epispore as hyaline, but in the type specimens studied it appeared to be reddish-brown. Also, the range of variation in size of the resting spores given by Spegazzini is less than that reported above.

By comparison the sporangial galls of *S. modioliensis* on fixed and stained leaf sections of *M. caroliniana* vary from 72–122 × 96–160 μ. As noted previously by the author [3] they vary somewhat in shape, but

[3] Karling, J. S. 1954. The galls of *Synchytrium modioliensis*. Bull. Torrey Bot. Club **81**: (In press).

like those of *S. australe* they are large and protruding, predominantly composite and multicellular with a sheath of cells around the infected one, and asymmetrical in relation to the subapical opening. In the living condition they are usually dark lavender-red or dubonnet in color. In fixed and stained preparations the sporangial sori vary from 48 to 89 μ in diameter. The sporangia vary from polyhedral, spherical, 18–42 μ, oblong, 21–32 × 38–53 μ, to slightly reniform, elongate and irregular in shape, with yellowish-orange, finely granular content and a hyaline wall. The resting spore galls are predominantly simple and unicellular and usually consist only of an enlarged epidermal cell. They may be spherical, 45–89 μ, oval, 68–85 × 87–120 μ, oblong, somewhat dome-shaped and subhemispherical with the lower part embedded in the palisade or mesophyll parenchyma. The upper portion may protrude slightly or prominently above the surface of the leaf. The resting spores are predominantly oval, 42–48 × 45–68 μ, to almost spherical, 32–65 μ, with a thick brown wall and coarsely granular content. These dimensions of the sporangial galls, sori and sporangia are considerably larger than those given by Cook, who listed these structures as 18 × 24 μ, 14 × 18 μ, and 3½ × 7 μ, respectively. His measurements or calculations appear to be incorrect because in prepared slides which he kindly sent the author shortly before his death the galls, sori and sporangia are markedly larger.

It is evident from this comparison that both species are long-cycled and produce sporangial and resting spore galls which are similar in structure. These galls, sori, sporangia, and resting spores are somewhat larger in *S. modioliensis*, but this difference may be due to shrinkage from drying in *S. australe* which was collected almost three quarters of a century ago. In view of the wide range of variation exhibited by both species these differences may not be significant. Furthermore, the initial hosts on which they were collected are reported to be identical. According to the Index Kewensis, Spegazzini [4] and Rodrigo [5] *Modiola prostrata* is synonymous with *M. caroliniana*, while Schumann [6] and Gleason [7] regard *Modiola* as a monotypic genus consisting of *M. caroliniana*. Whether or not the latter view is correct, most students of the

[4] Spegazzini, C. 1905. Flora de la provincia de Buenos Aires. Biedma i hijo, Buenos Aires, pp. 96–118.

[5] Rodrigo, A. de Pilar. 1938. Sinopsis de las malvacos bonariensis. Rev. Argentina Agron. **5**: 87–102.

[6] Schumann, K. 1895. Malvaceae. Engler and Prantl, Die Naturl. Pflanzenf. III, Abt. VI: 38.

[7] Gleason, H. A. 1952. The New Britton and Brown Illustrated Flora **2**: 532. New York.

Malvaceae agree that *M. multifida*, *M. prostrata* and *M. caroliniana* are identical.

Accordingly, on the basis of similarity in structure, size, shape and color of the galls, sori, sporangia and resting spores as well as identity of initial hosts, the author believes that *S. australe* Spegazzini and *S. modioliensis* Cook are the same species, and that on grounds of priority the latter should be listed as a synonym of the former. This interpretation is strengthened by Dr. Lindquist's recent (1953) collection of a *Synchytrium* species on *M. caroliniana* (*M. prostrata*) in Argentina which he sent to the writer. This species is the same as *S. australe* and as Cook's fungus so far as can be determined from herbarium specimens.

It should be emphasized in this connection, however, that although two species may appear to be identical in structure, size and shape of the galls, sori, sporangia and resting spores, the question of their identity cannot be settled completely until their life cycles are fully known. Species of *Synchytrium* are short- or long-cycled and vary in the presence or absence of a prosorus and the manner of resting spore germination, differences which distinguish the various subgenera. Prosori, for instance, occur in the life cycle of Cook's fungus, but the presence of such a developmental phase is difficult to determine with certainty in herbarium material like Spegazzini's type specimens. Also, germination of the resting spores has not been observed in *S. australe* or Cook's fungus, and it is not altogether improbable that they may differ in this respect. Obviously, this cannot be determined in Spegazzini's specimens because the resting spores are no longer viable.

The fungus collected by Jennings and Bartholomew on *Callirrhoë involucrata* may be the same as, or a biological race of *S. australe*. As will be reported in another paper, *S. australe* from Louisiana has a wide host range and can be transferred to a large number of malvaceous genera. It is quite possible that *C. involucrata* is one of its hosts in nature. Nevertheless, the identity and host range of the parasite on *C. involucrata* in Texas and Kansas are being investigated.—JOHN S. KARLING, Purdue University, Lafayette, Indiana.

ON THE DATES OF FUCKEL'S SYMBOLAE

The *Symbolae Mycologicae* of Leopold Fuckel and three *Nachträge* were published in the Jahrbücher des Nassuischen Vereins für Naturkunde, a serial issued in double volumes and with two years appearing on each title-page. The dates of publication have therefore been am-

biguous; most authors have either copied the double date or have accepted the earlier of the two years given for each part.

The *Symbolae* proper take up the whole of vol. **23–24** (pp. 1–459, *pl. 1–6*), dated 1869–70. On p. 91, l. 34, is cited Hedwigia **8**: 179. 1869, published in the twelfth and last part of its volume. On p. 238, l. 3, is cited a paper by Fuckel published in Bot. Zeit. **28**: 107, on Feb. 18, 1870. And in Jahrb. Nassau. Ver. Naturk. **25–26**: 473, l. 4, 5, it is stated that "Jahrgang 1869 und 70 wurde in 1870 . . . fertig." The date for the *Symbolae* is therefore 1870, somewhat later than Feb. 18.

The *Erster Nachtrag* appeared in vol. **25–26**, pp. 287–346, dated 1871–72. A separate at hand (repaged) bears the date 1871 on the title-page. On p. 473, l. 5, of that volume it is stated that "Jahrgang 1871 und 72 wird in etwa 8 wochen fertig"—this in a report delivered July 15, 1871. Page 478 of this volume, however, bears the date Nov. 1, 1871. Nachtr. 1 could then not have appeared in the complete volume before that date, but may well have been issued in advance; the year 1871 on its title-page must be accepted as correct.

The Zweiter Nachtrag appeared in vol. **27–28**, pp. 1–99, *1 pl.*; that volume is dated 1873–1874. The separate is dated 1873. On p. 228, l. 13–15, it is stated that "ist bis jetzt eine Abhandlung von Herrn Fuckel, Symbolae mycologicae, Nachtr. II, die ich Ihnen im Separatabdruck vorlege, fertig geworden"—this in a report submitted June 21, 1873. The date for this paper is then earlier than June 21, 1873.

The Dritter Nachtrag appeared in vol. **29–30**, pp. 1–39; the volume is dated 1875–1876. On p. 451, l. 11, 14, of that volume it is stated, under date of Nov. 20, 1875, that "ein dritter Nachtrag zu den Symbolae mycologicae von Herrn Fuckel . . . [ist] bereits fertig gedruckt." Presumably this means that separates of Nachtr. III (and not merely that part of the volume containing Fuckel's paper) were printed (and, it may be assumed, issued) before Nov. 20, 1875.

The *Symbolae* therefore appeared in the second of the two years on the title-page; each of the *Nachträge* on the first.—DONALD P. ROGERS, The New York Botanical Garden.

A NEW MYCOLOGICAL PERIODICAL

The Finnish Mycological Society (Suomen Sieniseura) has issued two numbers of KARSTENIA, described as a "journal of scientific and practical mycology," and expects to publish others about every other year. Number I (1950) in its 92 pages includes an article on P. A. Karsten (in Finnish), a long paper on poisonous fungi (Finnish, with

full English summary), one on fungi of the skin (English), one on wood-rotting fungi (Swedish, with good English summary), and notes on wood decay (Finnish, with German translation), *Paxillus* (German), rare fungi (Swedish, with English translation), *Isaria* on man (English, with Finnish summary), and other matters. The contents of number II (64 pages, 1953) are equally general, and equally available to those who do not read Finnish.

Dr. R. Tuomikoski, Botanical Institute, Unionink. 44, Helsinki, Finland, has expressed a willingness to send KARSTENIA in exchange for mycological journals or to individual mycologists in exchange for mycological reprints.—D. P. R.

REVIEWS

BIBLIOGRAPHIE DER PFLANZENSCHUTZ-LITERATUR, 1940–1945, by J. Bärner. 2 vols. xlviii + 1308 pp. Biologische Zentralanstalt für Land- und Forstwirtschaft in Berlin-Dahlem. Verlag Paul Parey, Berlin. 1953. Price not stated.

With the flood of papers that is being produced today, any tool that enables the investigator to keep abreast of the literature in his field is very welcome. Hence, the renewal of publication of the Bibliographie der Pflanzenschutz-Literatur should be well received by both phytopathologists and mycologists, for the material covered is not only that of plant diseases but also deterioration of plant materials by fungi and insects.

The content is well arranged; first, by cause and later by host. These parts are followed by a section on control measures and another on bibliographies. The field seems thoroughly covered with a minimum of duplication. The whole is summarized by an 88 page author-index. —JOSEPH C. GILMAN.

LES CHAMPIGNONS. TOME I. PHYSIOLOGIE, MORPHOLOGIE ET DEVELOPPEMENT. (ENCYCLOPÉDIE MYCOLOGIQUE XXII), by Fernand Moreau. xv + 940 pp., 465 figs. Paul Lechevalier, Paris. 6e. 1953. Price 11,000 fcs. (About $31.00.)

It is the expressed aim of the author of this imposing work to bring together all the significant facts necessary to present a completely modern view of the fungi. This makes it necessary to consider it somewhat more critically than if it purported to be merely another text. Furthermore, since the second volume, to be devoted to a systematic review of the fungi, has not yet appeared, any review must be hampered by having to consider the work as a part, rather than as a unified whole. Finally, in the case of so comprehensive an undertaking, it will take years of study and use to appraise its significance in the development of the science of mycology as a whole.

After a short and rather elementary introductory chapter, the remaining thirteen chapters (436 pages) constituting Part I are devoted to physiology, including discussion of nutrition, metabolism, symbiosis and mycotic infections. Part II, on morphology and development, includes

seventeen chapters (490 pages) covering such topics as fundamental morphology, cytology, the formation and dispersal of spores and their germination, sexuality and heterothallism, and genetics. Each chapter and, in the case of the longer chapters, each chapter section, has its own bibliography. There is no index. Presumably this will appear in the second volume. Until it appears, the use of the first volume as a source of reference will be seriously handicapped.

There can be no question as to the enormous amount of information assembled in this volume. The classical literature is well covered, as is modern French literature. Other modern literature, especially American, is less adequately recognized. The author tacitly acknowledges this in his preface. The result is a curious mixture of advanced and rather obsolete views. Examples of the latter are the treatment of the lichens; the reference to the germination of a rust teliospore where each cell of the "promycelium" is said to produce "une conidie, appelée sporidie." Less important, but even more obvious are such anachronisms as *Boletus edulis* "Fries ex Bull." and *Agaricus "campester* Fries ex Linné." Why, in dealing with Basidiomycetes, modern French authors should adopt the obsolete classification and terminology of Brefeld rather than the far superior treatment of Patouillard, continues to be a mystery. There is an excellent discussion of mechanisms for spore liberation, but no reference to recent work on the splash-cup adaptation nor to the effect of rain on spore dispersal in *Lycoperdon* and similar genera.

The significant contributions of French mycologists have, until recent years, been unduly neglected in English-speaking countries and particularly in the United States. The recent appearance of this and several other large and important modern French works which have been noted in these columns, should do much to remedy this situation. The book under consideration is too extensive for use as a text and the unavoidable high price will make its purchase difficult for all but a small minority of students. Its place is obviously on reference shelves, where it promises to be useful for many years to come.—G. W. MARTIN.

UREDINEANA. VOL. IV. (ENCYCLOPÉDIE MYCOLOGIQUE XXIV), ed. by A. L. Guyot. 602 pp. Paul Lechevalier, Editeur, Paris. 6e. 1953. Price 9,000 fcs. (About $25.75.)

Uredineana appears intermittently as parts of the Encyclopédie Mycologique. The first volume appeared in 1938, the second in 1946 and the third in 1951. It is devoted to systematic studies and biology of the

rusts of the world and publication is open to all students of this group of fungi. It is printed on good paper; the photographs, drawings and editing are excellent. It appears to this reviewer that Uredineana should be available to all students of the plant rusts.

THE SPECIES OF PUCCINIA PARASITIC ON THE ANDROPOGONEAE, George B. Cummins, pp. 5–90, Pl. I–XI. The author presents a critical systematic study of 55 species of *Puccinia* which he recognizes on grasses of the tribe Andropogoneae including the related tribe Tripsaceae following the grass nomenclature of Pilger (Natur. Pflanzenf. **14**: 107–184. 1940). *Puccinia arthraxon-ciliaris* on *Arthraxon* spp., *P. erianthicola* on *Erianthus* spp., *P. kawandensis* on *Chrysopogon aucheri* and *P. pseudocesatii* on *Bothriochlon* and *Chrysopogon* are described as new species. The aecial stage is known of only seven of the species. Forty of the species are restricted to the "eastern hemisphere" in contrast to eight species present only in the western hemisphere. Seven species occur in both the eastern and western world of which three may be designated as world-wide in the warmer regions. Thirty species occur only in the northern hemisphere while only four species are known only in the southern hemisphere. Two previously recognized species are excluded by the fact that their hosts were incorrectly named and a third species excluded by reason of its transfer to another genus (*Phakopsora*) of the rusts. A key to the species is presented. Each species is illustrated by excellent microphotographs of the spores. American pathologists should recognize the danger of introducing into the Americas this relatively large number of rusts at present restricted to the old world.

PUCCINIASTREAE AND MELAMPSOREAE OF NORWAY, Ivar Joerstad, pp. 91–123. In the Pucciniastreae six genera are represented; *Hyalopsora* (2 species); *Melampsorella* (1 species); *Melampsoridium* (1 species); *Milesina* (5 species); *Pucciniastrum* (9 species); *Uredinopsis* (2 species). All twenty species are heteroecious with haploid stage on conifers although only one species, *Pucciniastrum areolatum* is obligatorily so. The others are independent of host-alternation. The haploid hosts of seventeen on these species have not been found in Norway. Five species occur as far north as 70°. Melampsoreae is represented by fourteen species of *Melampsora* and nine "races" or "race-groups" usually recognized as species but here regarded as belonging to *M. epitea* (5) and *M. populnea* (4), the former with diploid stage on *Salix* and the latter with diploid on *Populus*. Eight species are heteroecious, four are autoecious and

the life cycle of two species is unknown. Two species are completely independent of host alternation.

URÉDINALES D'AFRIQUE, 3RD NOTE. URÉDINALES DE CÔTE D'IVOIRE, 2ND NOTE, G. Viennot-Bourgin, pp. 125–228, figs. 1–41. This paper treats 39 species of rusts collected in the Ivory Coast by the author from July through September 1951. Twelve of the species are described by the author as new: *Goplana cissi* on *Cissus petiolata* and *C. polyantha* (type), Ivory Coast; *Hapalophragmium millettia-rhadenthae* on *Millettia rhodantha*, Ivory Coast; *Hemileia mussaendae* on *Mussaenda erythrophylla*, Ivory Coast; *Puccinia baphiae* on *Baphia pubescens*, Ivory Coast; *Puccinia digitariae-velutinae* on *Digitaria velutina*, Ivory Coast; *Puccinia virectae* on *Virecta multiflora*, Ivory Coast; *Sphaerophragmium millettiae* on *Millettia stappiana*, Ivory Coast; *Sphaerophragmium monodorae* on *Monodora tenuifolia*, Ivory Coast; *Uredo bosquiae* on *Bosquiea angolensis*, Ivory Coast; *Uredo markamiae* on *Markamia lutea*, Ivory Coast; *Uredo newbouldiae* on *Newbouldia laevis*, Ivory Coast; *Uromyces aspiliella* on *Aspilia latifolia*, Ivory Coast. Several of the species collected were unknown previously in Africa. The illustrations are excellent and the descriptions are fully adequate. A critical bibliography is appended.

CONTRIBUTIONS A L'ÉTUDE DE LA FLORE URÉDINOLOGIQUE QU SUD-OUEST ET DES PYRÉNÉES (FRANCE), 4TH NOTE, G. Dupias, pp. 229–239. This is a list of fifty-six species of rusts collected in southwest France and the Pyrenees distributed by genera as follows: *Aecidium* (2), *Chrysomyxa* (1), *Coleosporium* (2), *Endophyllum* (2), *Gymnosporangium* (4), *Melampsora* (6), *Puccinia* (24), *Uromyces* (15).

URÉDINÉES RECOLTÉES DANS LA RÉGION MEDITERRANÉENNE FRANCAISE, G. Dupias, pp. 241–244. The author records 31 species of rusts, with brief notes, collected on a trip in the Maritime Alps and the western Pyrenees of France.

SUR DEUX UROMYCES PARASITES DES LÉGUMINEUSES, G. Dupias, pp. 245–248. *Uromyces lereddei* Dupias nov. sp. is described from the Gallic Pyrenees on *Colutea arborescens* which the author considers distinct from *U. coluteae* Arth. on the same host, as well as other related species on closely related hosts. Additional descriptive details are given regarding *Uromyces mangenoti* Mayor and V.-B. on *Vicea pubescens*.

LES DEUX ROUILLES DU COMPAGNON BLANC: *Melandryum album* (Mill.) Garcke., G. Kuhnholtz-Lordat, pp. 249–252, figs. 1–3. The article contains notes on teliospore germination of *Puccinia arenariae* (Schum.) Wint. which occurred from June to August. Attention is called to differences in thicknesses of the apex of the teliospore of *Uromyces verruculosus* Schroet. as described by various workers, and the presence in July at Montpellier of abundant telia, which are rarely seen in this species.

LES ROUILLES DE L'OGAT: *Ammophila arenaria* Link, A. L. Guyot, pp. 253–256, fig. 1. The article contains notes and comparative descriptions of *Puccinia ammophilae* (Syd.) Guyot and *P. ammophilina* (Kleb.) Mains.

LES ROUILLES DÉFORMANTES DE L'ORIGAN ET DU SERPOLET, A. L. Guyot, M. Massenot, and J. Bulit, pp. 257–267, figs. 1–6. This paper is a critical study of *Puccinia caulincola* Schneid. and *P. rubsaameni* Mag., both microcyclic rusts causing witches brooms on Labiatae, the former on *Thymus* spp., the latter on *Origanum vulgare*. Their geographical distribution is similar, but their teliospore measurements statistically distinct.

LES ROUILLES COURONNÉES DES FÉTUQUES, A. L. Guyot, M. Massenot, pp. 269–279, tabls. 1–3. The authors present statistical evidence in justification of the independence of three coronate species of *Puccinia* inhabiting species of *Festuca*, i.e., *P. coronata* Corda., *P. festucae* and *P. gibberosa*.

ÉTUDE EXPÉRIMENTALES SUR LES URÉDINÉES HÉTÉROÏQUE, A. L. Guyot and M. Massenot, pp. 281–353, figs. 1–15, Tabls. I–XIV. The authors inoculated 24 species of grasses of the genera *Aegilops, Agropyron, Bromus, Elymus, Haynaldia, Hordeum, Secale* and *Triticum* with aeciospores from *Clematis flammula, C. recta* and *C. vitalba* collected in Southern France. No infection resulted on four species of *Bromus* or on *Triticum vulgare*. Uredia from the resulting infections were used for cross inoculation on species of other genera. *Hordeum maritimum* proved to be the most congenial host for all forms of the rusts on *Clematis*. The following cases of heteroecious specialization were

proved: *P. agropyri* on *A. campestri* to *Clematis flammula; P. altermans* on *Bromus erectus* to *Thalictrum minus; P. arrhenatheri* on *A. bulbosum* to *Berberis vulgaris; P. coronata* on *Arrhenatherum bulbosum, Bromus erectus* and *Elymus virginium* to *Rhammus cathartica; P. cynodontis* on *C. dactylon* to *Plantago lanceolata; P. madritensis* on *Bromus sterilis* to *Clematis alpina* and *C. flammula; P. persistens* on *Agropyron repens* to *Thalictrum flavens; P. phragmites* on *P. communis* to *Rumex obtusifolius; P. sessilis* on *Phalaris arundinacea* to *Arum maculatum; P. triticina* on *Triticum spelta* to *Thalictrum flavum.* Twenty-three collections of aecia on *Euphorbia cyparissias* were used as the source of aeciospore inoculations on species of *Cytissus, Genista, Lathyrus, Medicago, Pisum, Trifolium* and *Lotus.* Of these four proved to be *Uromyces genistae-tinctoriae,* fifteen belonged to *U. striatus,* one to *Uromyces loti,* and three to *U. pisi.* Two collections of aecia on *Euphorbia gerardiana* and two collections on *E. nicaeensis* proved to be stages of *Uromyces caryophyllinus.* The paper is of special interest to taxonomists and students in the specialization of the rusts.

CONTRIBUTION A L'ÉTUDE DES URÉDINÉES PARASITES DE LA FLORA TUNISIENNE, A. L. Guyot, pp. 355–372, figs. 1–2. Notes and descriptions of 18 species of rusts previously unreported from Tunisia.

REVUE BIBLIOGRAPHIQUE REGIONAL, pp. 373–504. This large section appears in two parts. The *first* is a list of the rusts and hosts collected in Central Asia and recorded by W. Tranzxchel in 1939 (Conspectus Uredinalium, U.S.S.R.) with a brief introduction covering the climatic, physiographic and floristic features of the countries. The *second* is a selected and abstracted bibliography of publications on the plant rusts covering the eastern hemisphere chiefly appearing during the last decade.

CORRECTIONS, ADDITIONS ET OBSERVATIONS, A. L. Guyot, pp. 505–552, figs. 1–3. Notes on 53 species of the genus *Uromyces.*

NOTES. Pp. 523–527. General notes on miscellaneous rusts.

INDEX. Pp. 529–602. By species of rusts and hosts.—C. R. ORTON.

DISEASES OF BRITISH GRASSES AND HERBAGE LEGUMES, by K. Sampson and J. H. Western. Second ed. xiii + 118 pp., 19 figs. Published for the British Mycological Society by the Cambridge University Press. 1954. Price, $3.00.

This comprehensive manual, carefully edited and beautifully printed, is about one-third longer than the first edition. As in that edition, it treats only of the diseases of evident economic importance in the British Isles but is written to be of aid to workers everywhere.

The nine plates of illustrations are well done and the nineteen text figures are adequate. A few of the latter could be improved. Text-fig. 7 (of *Passalora graminis*) of the second edition has replaced text-fig. 6 of the first edition, although the original was more useful.

The taxonomy is conservative but modern. The author's treatment of the virus diseases is admittedly still somewhat inadequate.

About 530 well selected references are included.

This manual is not too overpriced. It should be included in the bookshelf of all workers in the forage disease field and will be especially helpful to students.—RODERICK SPRAGUE.

MANUSCRIPT

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